Fig 1A

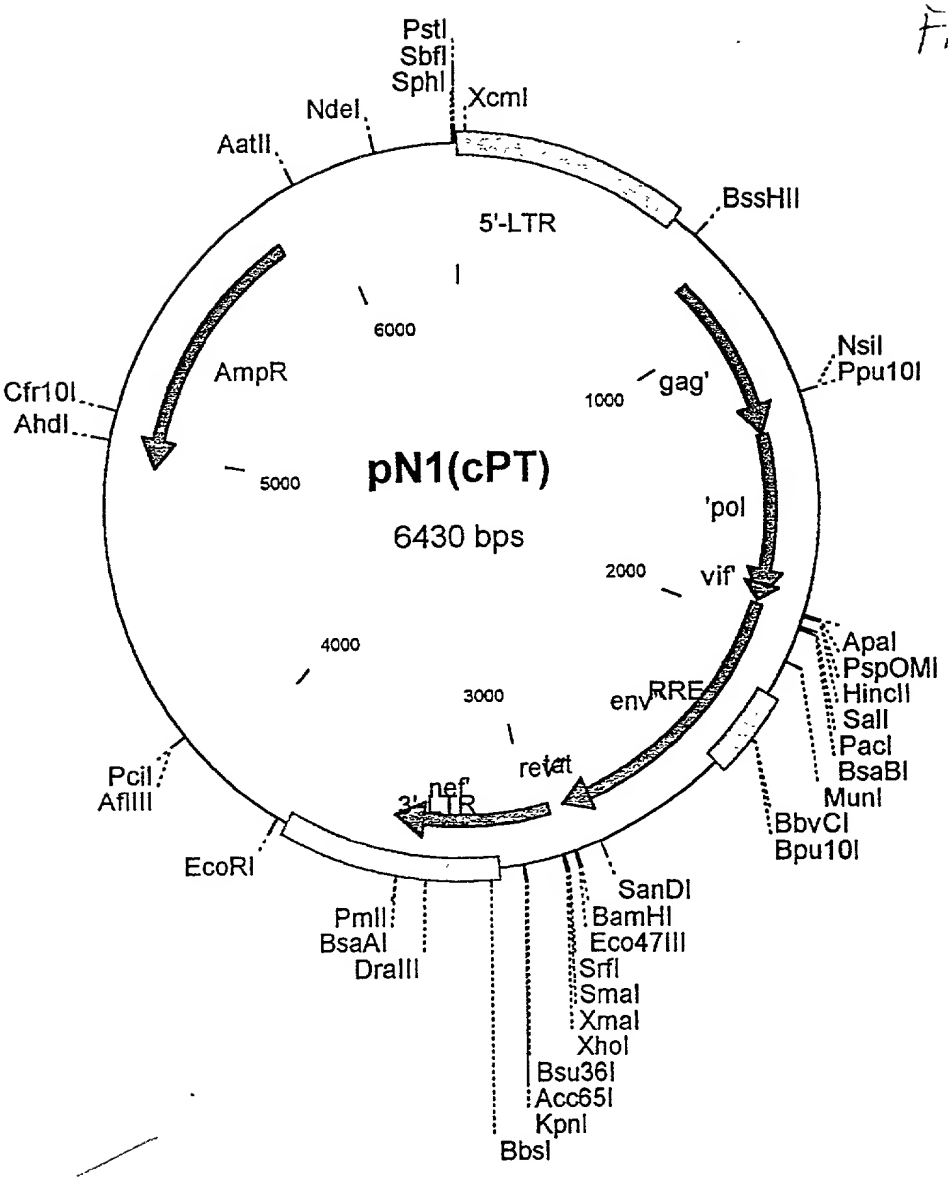

pN1(cPT)
6430 bps
5'-LTR
gag'
'pol
vif'
env
RRE
rev
tat
nef
3'-LTR
AmpR
1000
2000
3000
4000
5000
6000
PstI
SbfI
SphI
XcmI
NdeI
AatII
BssHII
NsiI
Ppu10I
Cfr10I
AhdI
ApaI
PspOMI
HincII
SalI
PacI
BsaBI
MunI
BbvCI
Bpu10I
SanDI
BamHI
Eco47III
SrfI
SmaI
XmaI
XhoI
Bsu36I
Acc65I
KpnI
BbsI
PmlI
BsaAI
DraIII
EcoRI
PciI
AflIII

Fig 1B

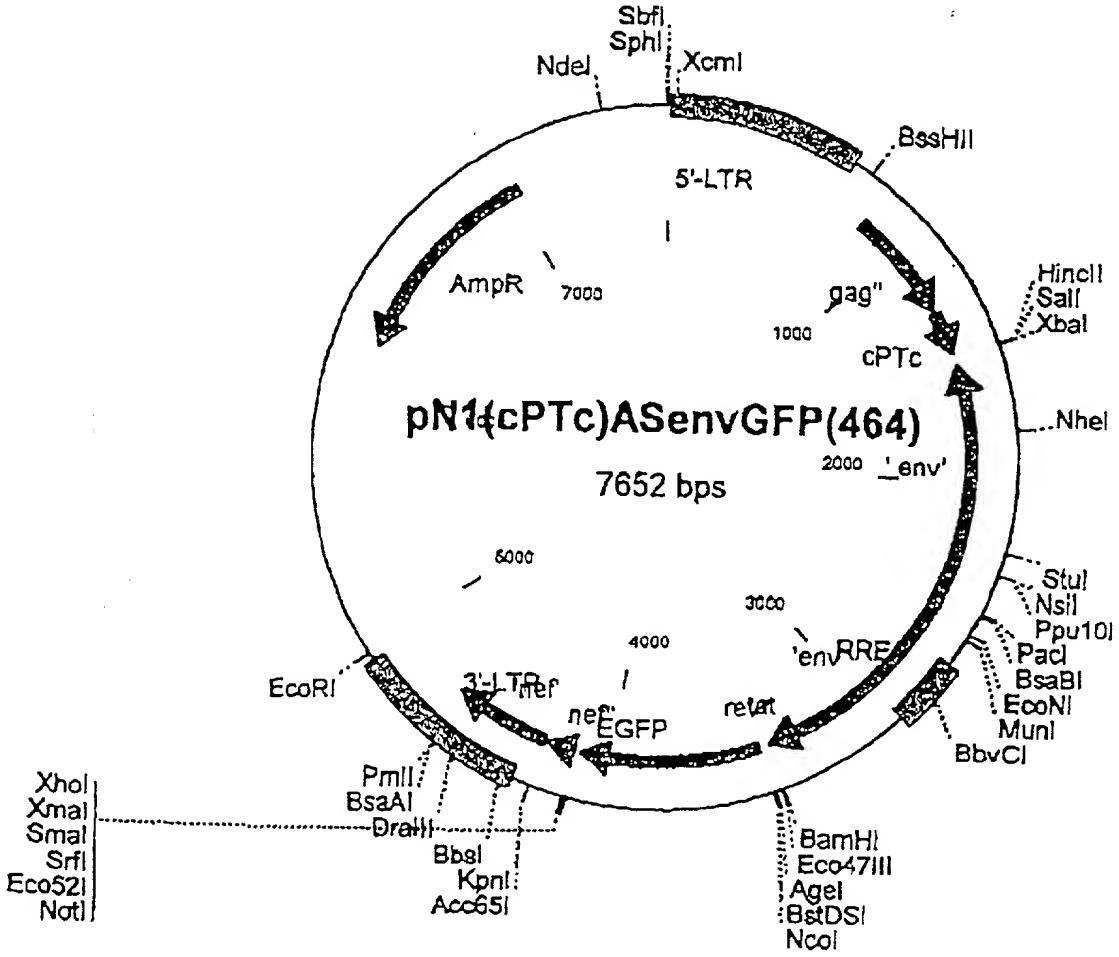

SbfI
SphI
NdeI
XcmI
BssHII
5'-LTR
AmpR
7000
gag''
1000
cPTc
HincII
SalI
XbaI
pN1(cPTc)ASenvGFP(464)
7652 bps
2000
'env'
NheI
6000
3000
4000
StuI
NsiI
Ppu10I
PacI
BsaBI
EcoNI
MunI
BbvCI
'envRRE'
EcoRI
3'-LTR
nef
EGFP
retart
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
BamHI
Eco47III
AgeI
BstDSI
NcoI

Fig 1C

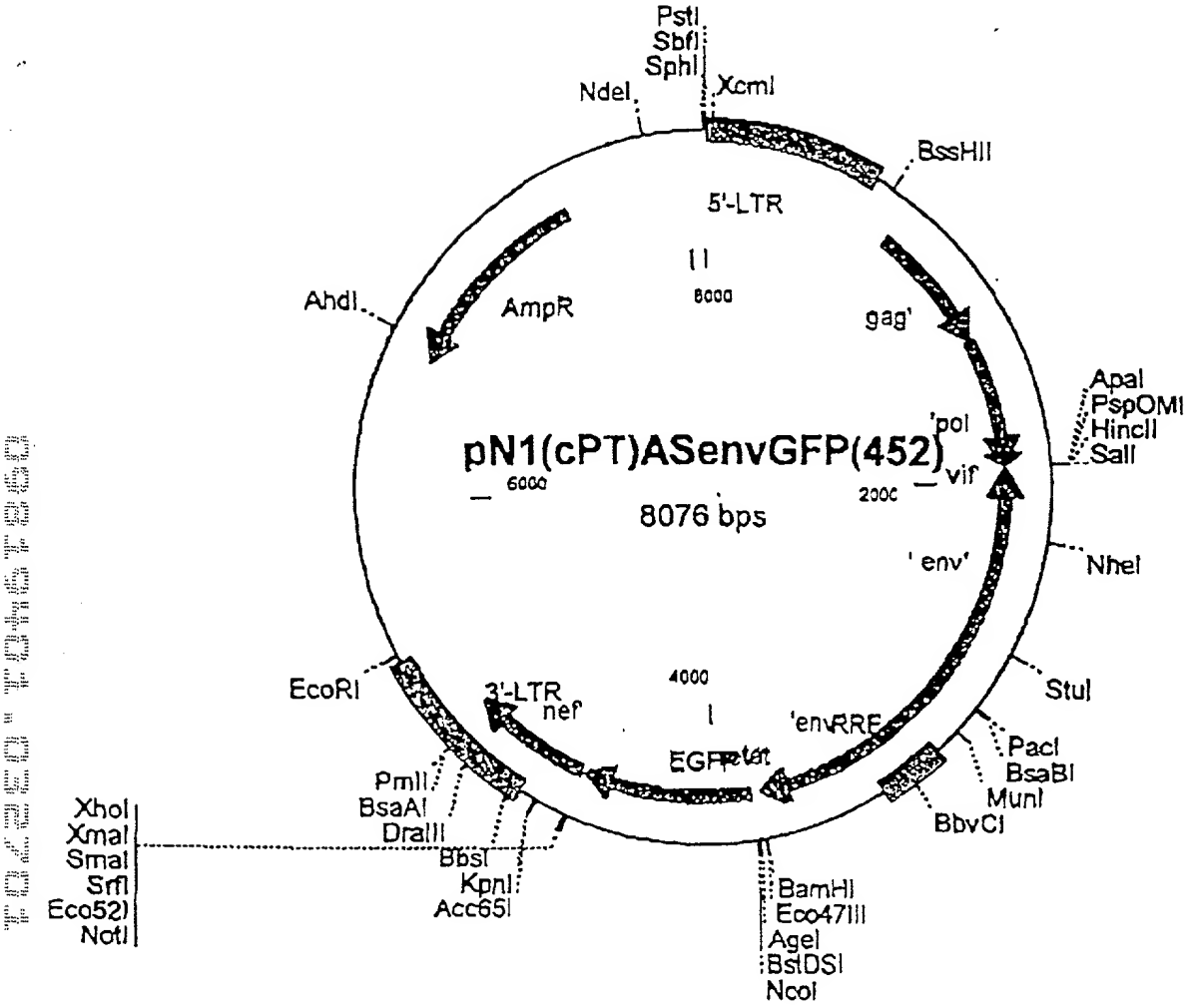

pN1(cPT)ASenvGFP(452)
8076 bps
PstI
SbfI
SphI
XcmI
NdeI
BssHII
5'-LTR
AhdI
AmpR
gag'
'pol
vif
ApaI
PspOMI
HincII
SalI
' env'
NheI
StuI
PacI
BsaBI
MunI
BbvCI
'envRRE
rev
EGFP
tat
3'-LTR
nef
EcoRI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BamHI
Eco47III
AgeI
BstDSI
NcoI
2000
4000
6000
8000

Fig 1

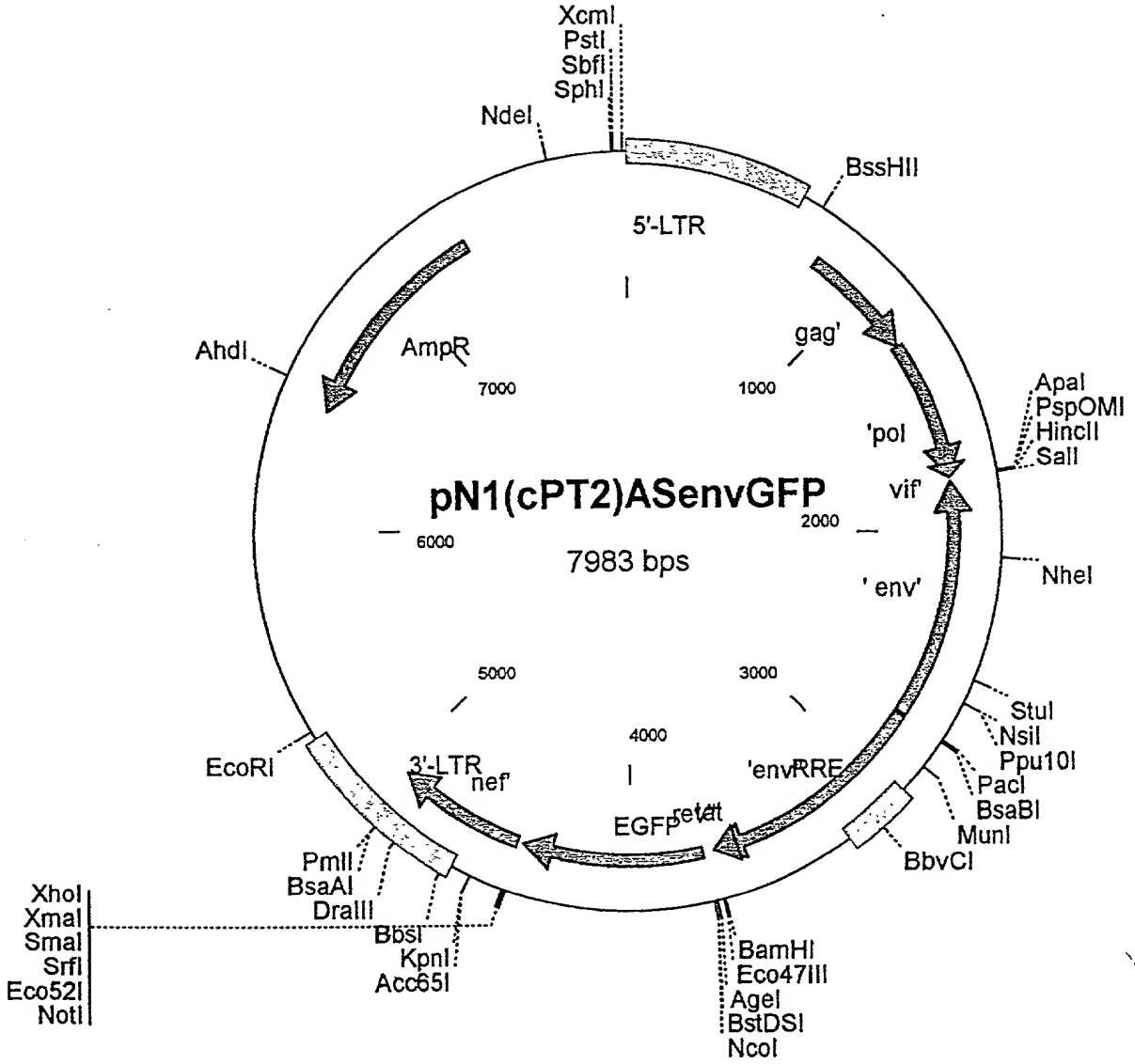

pN1(cPT2)ASenvGFP
7983 bps
5'-LTR
gag'
'pol
vif'
' env'
'envRRE
rev
tat
EGFP
nef'
3'-LTR
1000
2000
3000
4000
5000
6000
7000
XcmI
PstI
SbfI
SphI
NdeI
BssHII
ApaI
PspOMI
HincII
SalI
NheI
StuI
NsiI
Ppu10I
PacI
BsaBI
MunI
BbvCI
BamHI
Eco47III
AgeI
BstDSI
NcoI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
EcoRI
AhdI
AmpR

Fig 1E

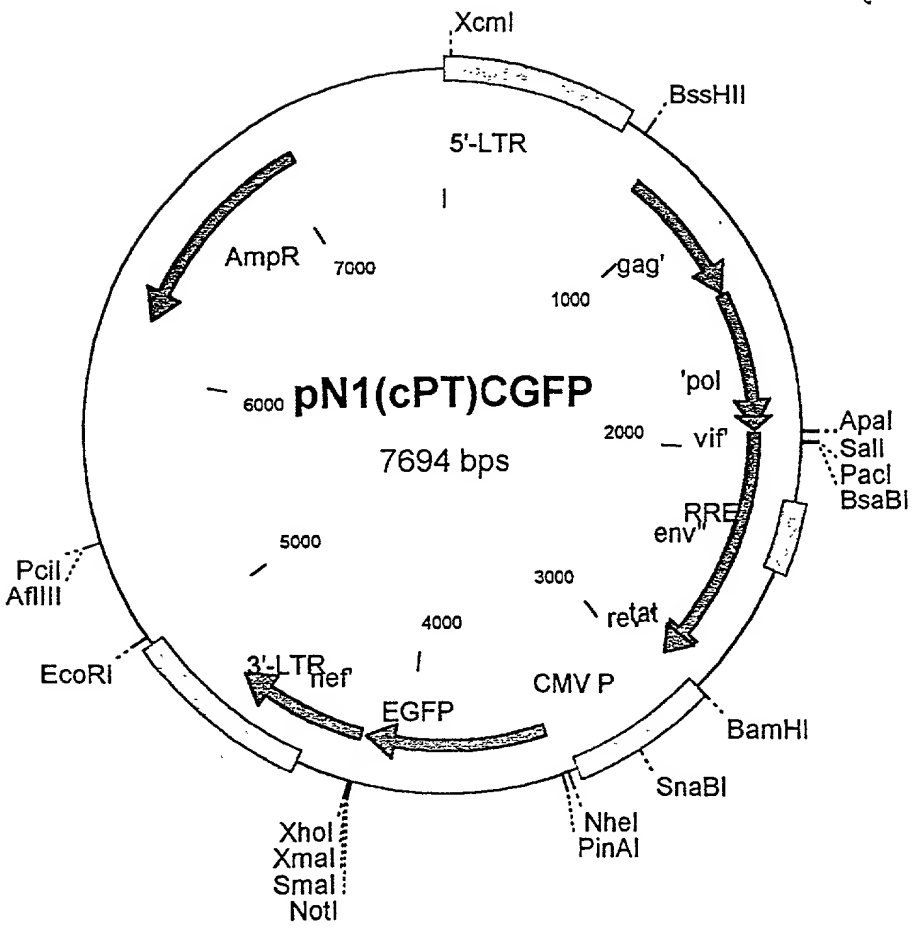

Xcml
BssHII
5'-LTR
AmpR
7000
gag'
1000
pN1(cPT)CGFP
7694 bps
'pol
2000
vif'
ApaI
SalI
PacI
BsaBI
RRE
env'
5000
3000
PciI
AflIII
rev'
4000
3'-LTR
EcoRI
CMV P
EGFP
BamHI
SnaBI
NheI
PinAI
XhoI
XmaI
SmaI
NotI

Fig 1F

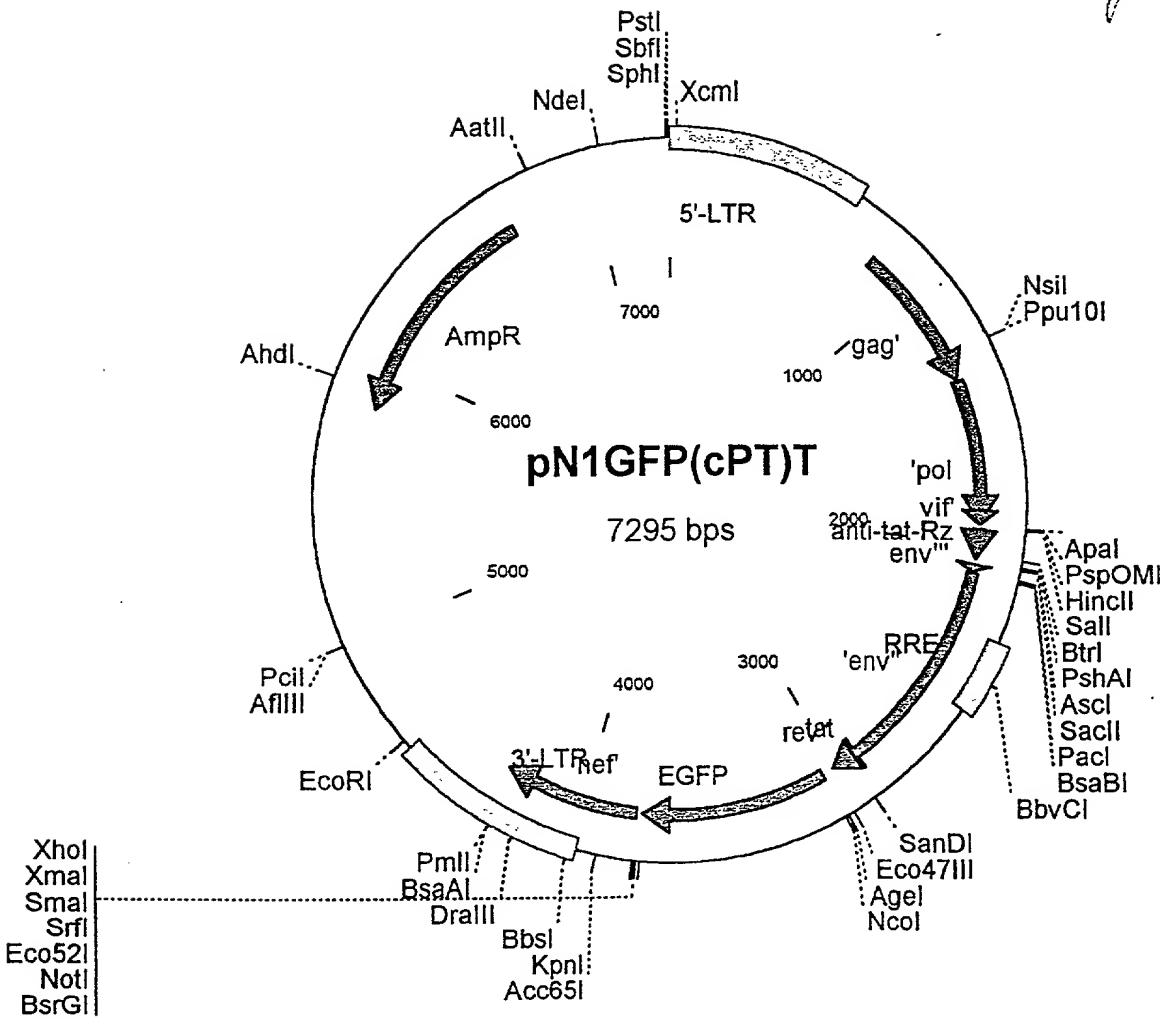

pN1GFP(cPT)T
7295 bps
5'-LTR
'gag'
'pol
vif'
anti-tat-Rz
env'"
'env'
RRE
rev
tat
EGFP
3'-LTR
nef'
AmpR
7000
1000
2000
3000
4000
5000
6000
PstI
SbfI
SphI
XcmI
NdeI
AatII
NsiI
Ppu10I
AhdI
ApaI
PspOMI
HincII
SalI
BtrI
PshAI
AscI
SacII
PacI
BsaBI
BbvCI
SanDI
Eco47III
AgeI
NcoI
PciI
AflIII
EcoRI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I

Fig 16

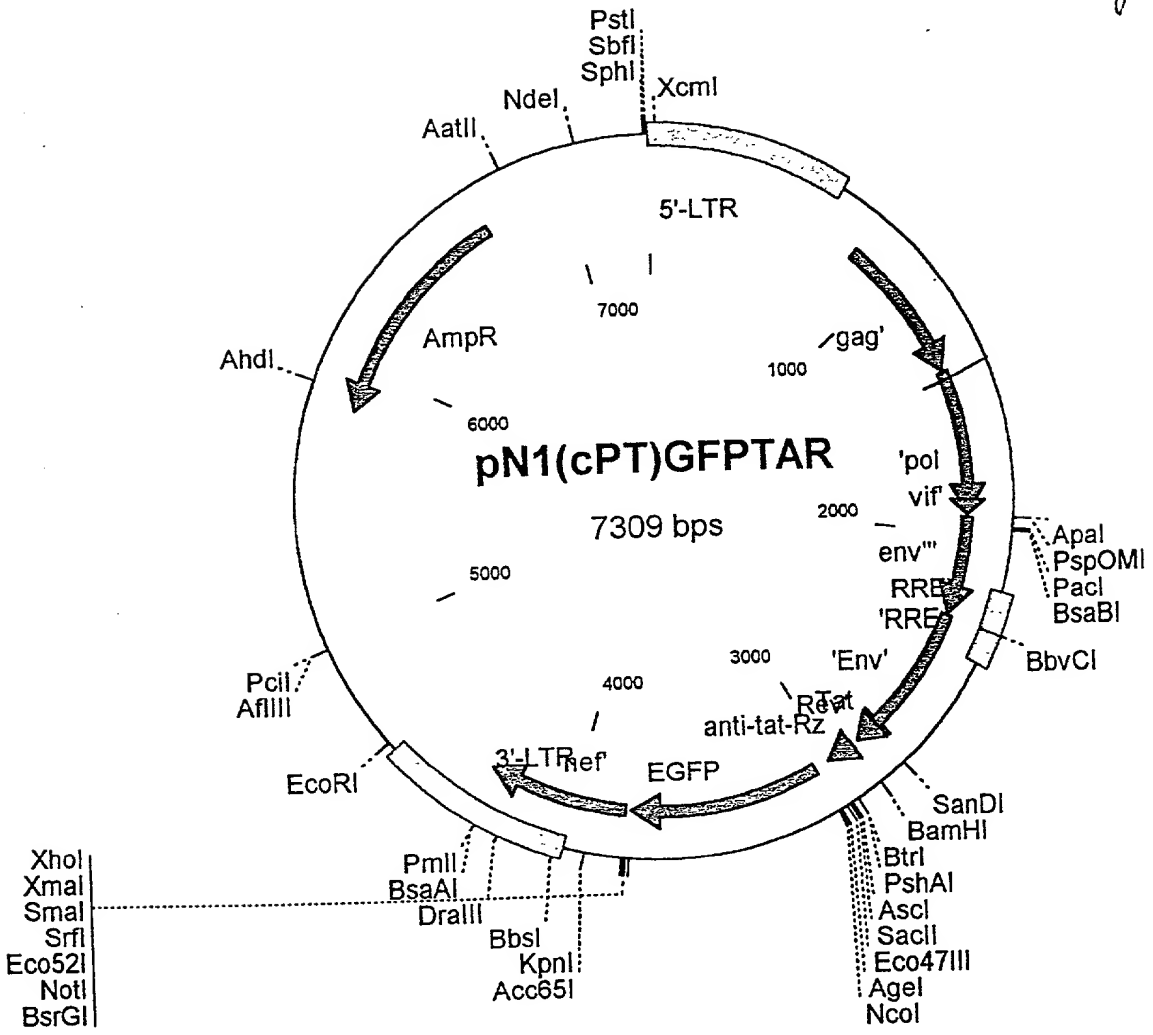

PstI
SbfI
SphI
XcmI
NdeI
AatII
5'-LTR
7000
AmpR
gag'
1000
AhdI
6000
pN1(cPT)GFPTAR
'pol
vif'
7309 bps
2000
ApaI
PspOMI
PacI
BsaBI
env'''
5000
RRE
'RRE
BbvCI
3000
'Env'
PciI
AflIII
4000
Rev
Tat
anti-tat-Rz
3'-LTR
nef'
EGFP
EcoRI
SanDI
BamHI
BtrI
PshAI
AscI
SacII
Eco47III
AgeI
NcoI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I

Fig 1H

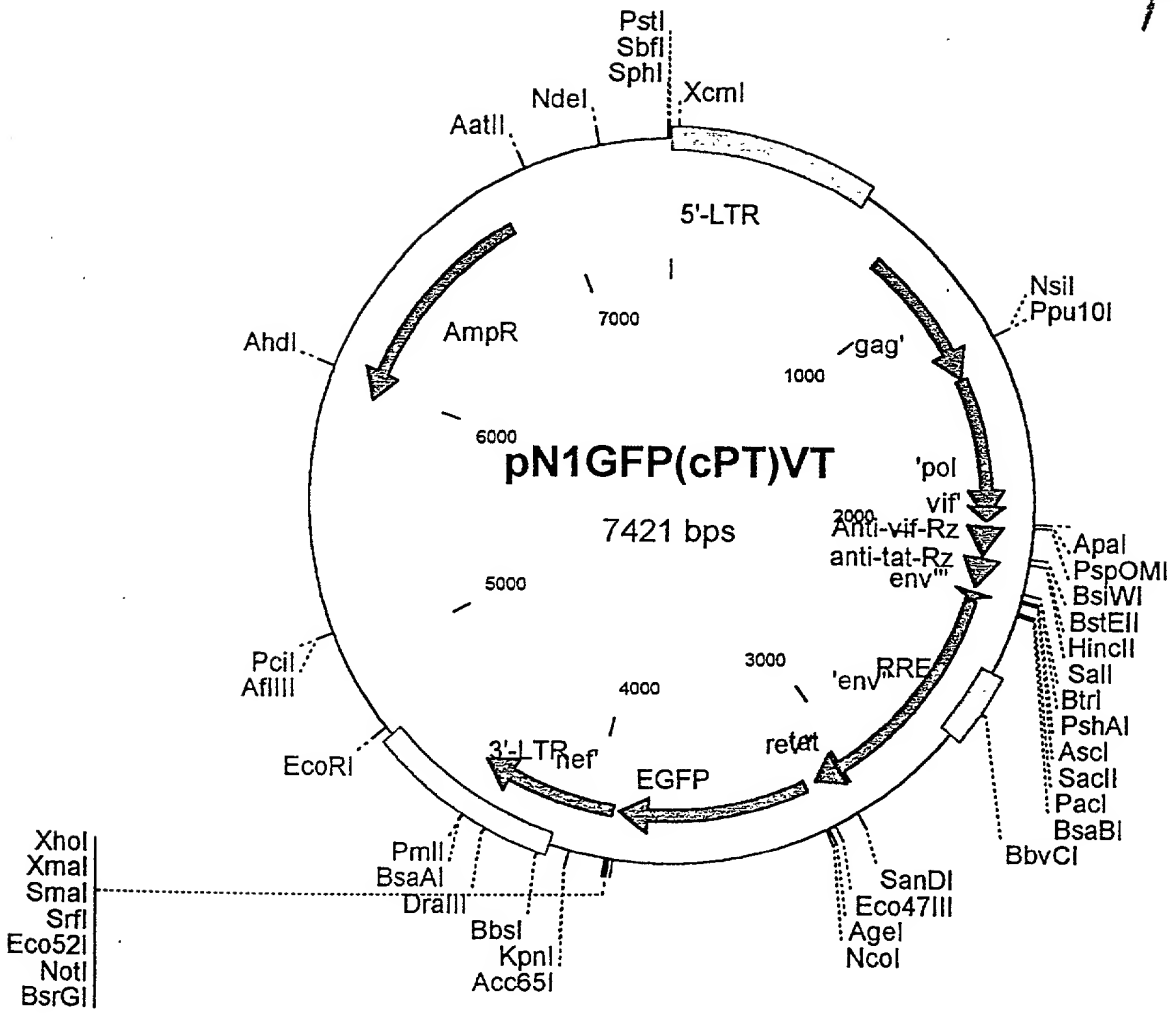

pN1GFP(cPT)VT
7421 bps
5'-LTR
AmpR
gag'
'pol
vif'
Anti-vif-Rz
anti-tat-Rz
env'''
'env
RRE
rev
EGFP
3'-LTR
nef'
1000
2000
3000
4000
5000
6000
7000
PstI
SbfI
SphI
XcmI
NdeI
AatII
NsiI
Ppu10I
AhdI
ApaI
PspOMI
BsiWI
BstEII
HincII
SalI
BtrI
PshAI
AscI
SacII
PacI
BsaBI
BbvCI
PciI
AflIII
EcoRI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
SanDI
Eco47III
AgeI
NcoI

Fig 1I

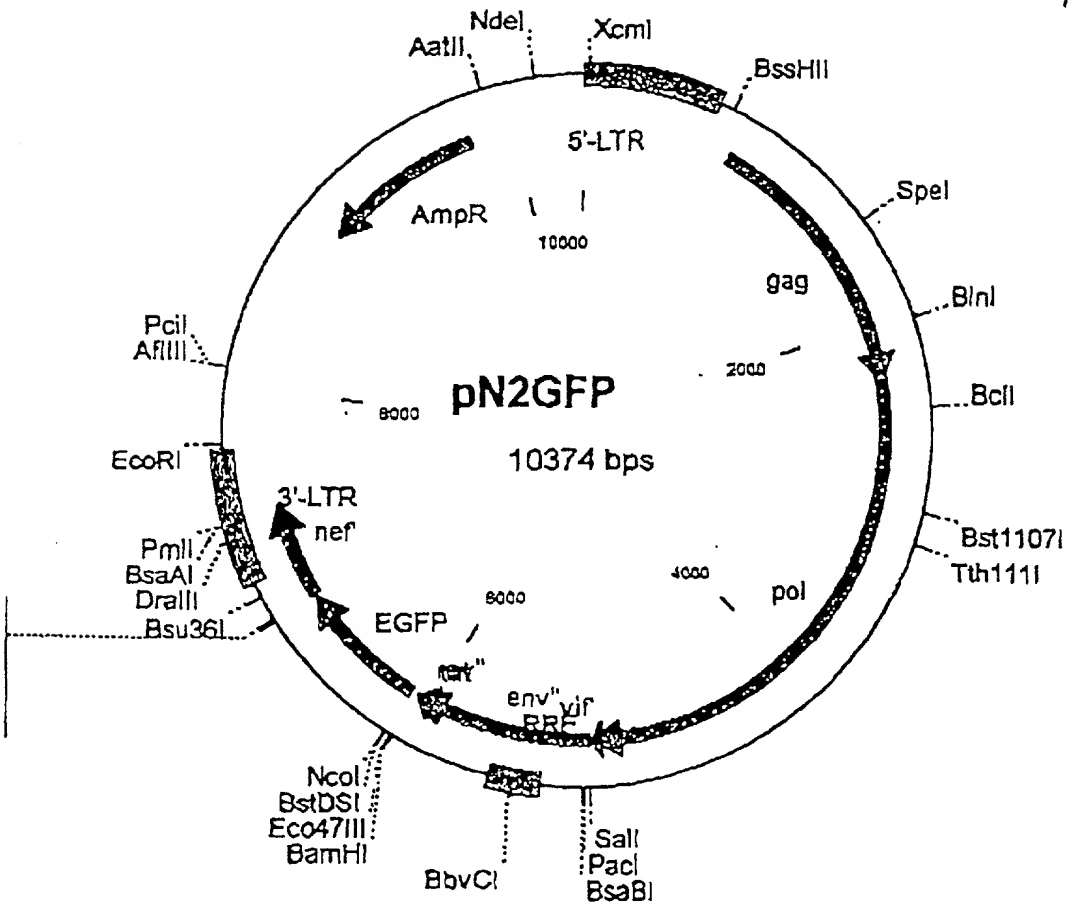

pN2GFP
10374 bps
5'-LTR
AmpR
gag
pol
env
rev
RRE
tat
nef
3'-LTR
EGFP
10000
2000
4000
6000
8000
NdeI
AatII
XcmI
BssHII
SpeI
BlnI
BclI
Bst1107I
Tth111I
PciI
AflIII
EcoRI
PmlI
BsaAI
DraIII
Bsu36I
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
NcoI
BstDSI
Eco47III
BamHI
BbvCI
SalI
PacI
BsaBI

Fig 1J

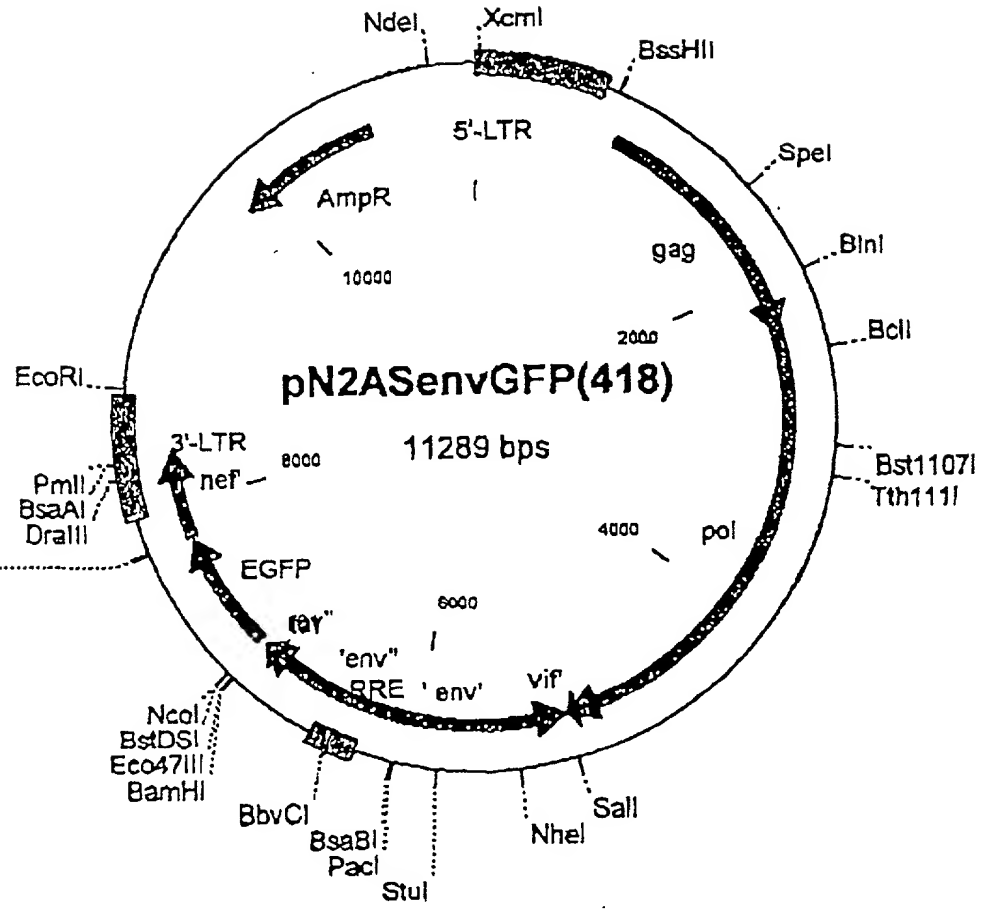
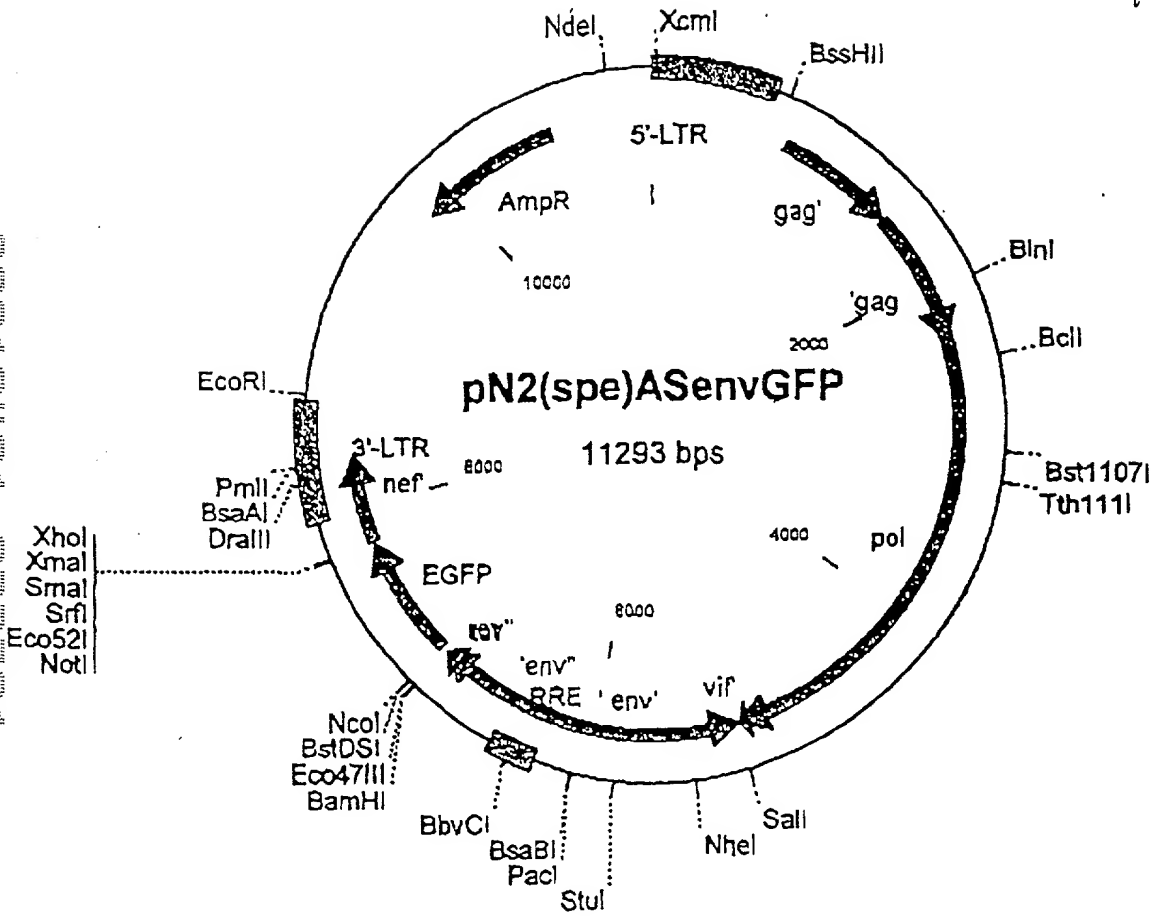

pN2ASenvGFP(418)
11289 bps
5'-LTR
AmpR
gag
pol
'env'
vif'
RRE
'rev'
EGFP
nef'
3'-LTR
2000
4000
6000
8000
10000
NdeI
XcmI
BssHII
SpeI
BlnI
BclI
Bst1107I
Tth111I
SalI
NheI
StuI
PacI
BsaBI
BbvCI
NcoI
BstDSI
Eco47III
BamHI
EcoRI
PmlI
BsaAI
DraIII
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI

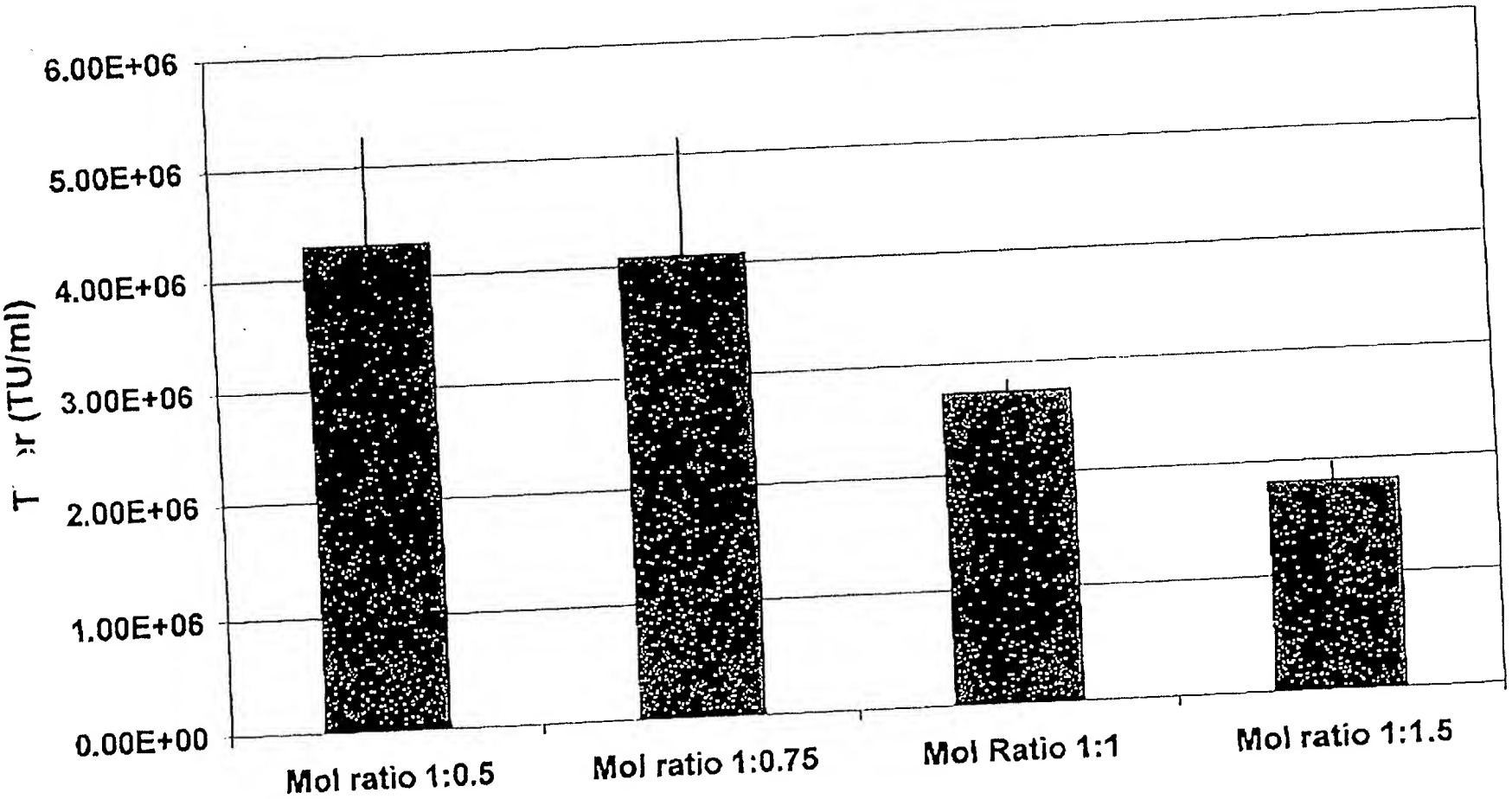
Fig 1K


pN2(spe)ASenvGFP
11293 bps
5'-LTR
AmpR
gag'
'gag
pol
'env'
vif'
RRE
'env"
tat"
EGFP
nef'
3'-LTR
NdeI
XcmI
BssHII
BlnI
BclI
Bst1107I
Tth111I
EcoRI
PmlI
BsaAI
DraIII
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
NcoI
BstDSI
Eco47III
BamHI
BbvCI
BsaBI
PacI
StuI
NheI
SalI
2000
4000
6000
8000
10000

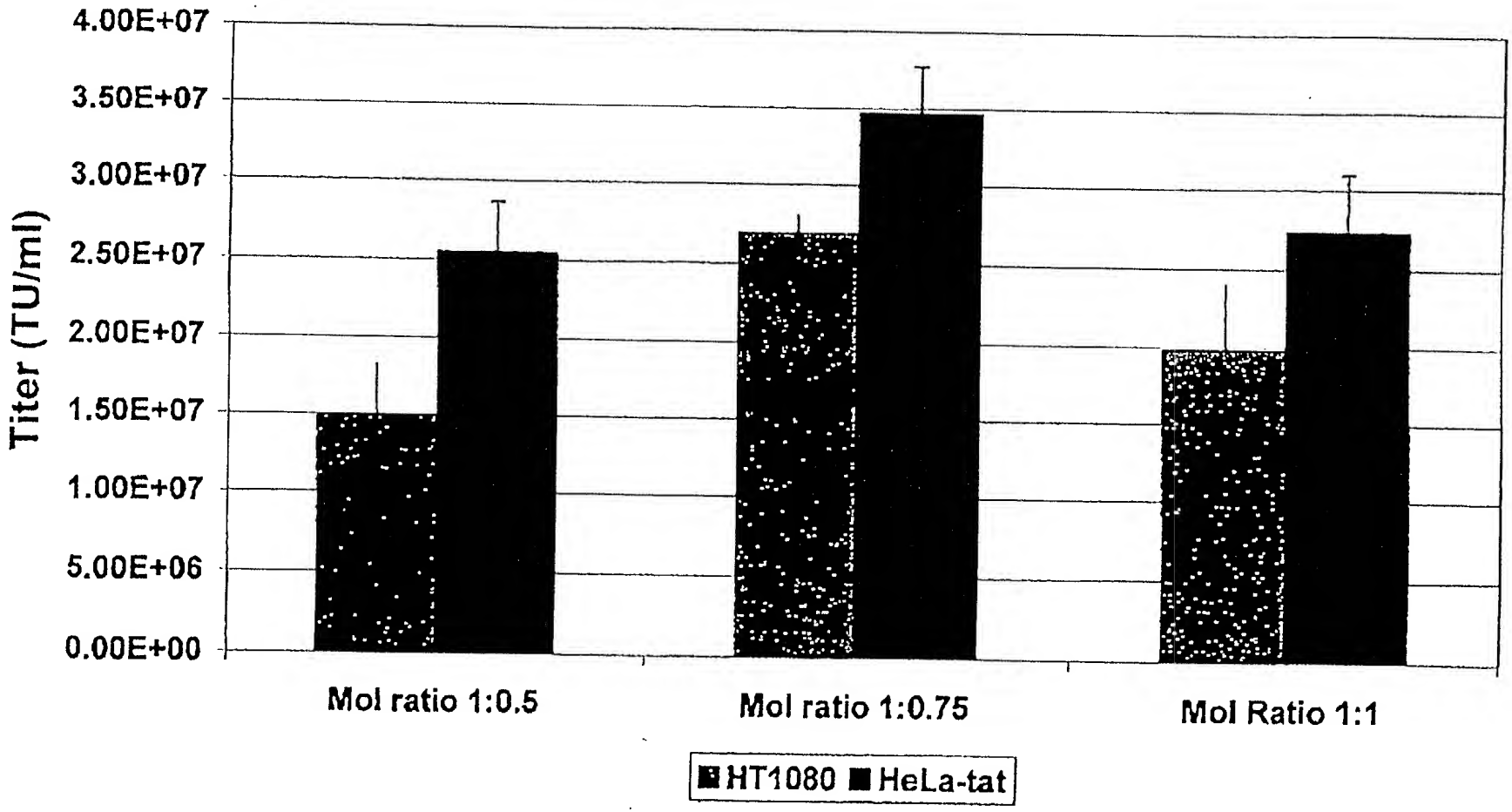
A	+105	GTGTGCCCGTCTG	+117
B		. . . . . .AC . . .	
A	+118	TTGTGTGACTCTG	+130
B		. . . . .	
A	+131	GTAACTAGAGATC	+143
B		.C.G. . . . . .A.	

FIG. 2

Ratio Optimization for pN1(cPTC)ASenvGFP Vector

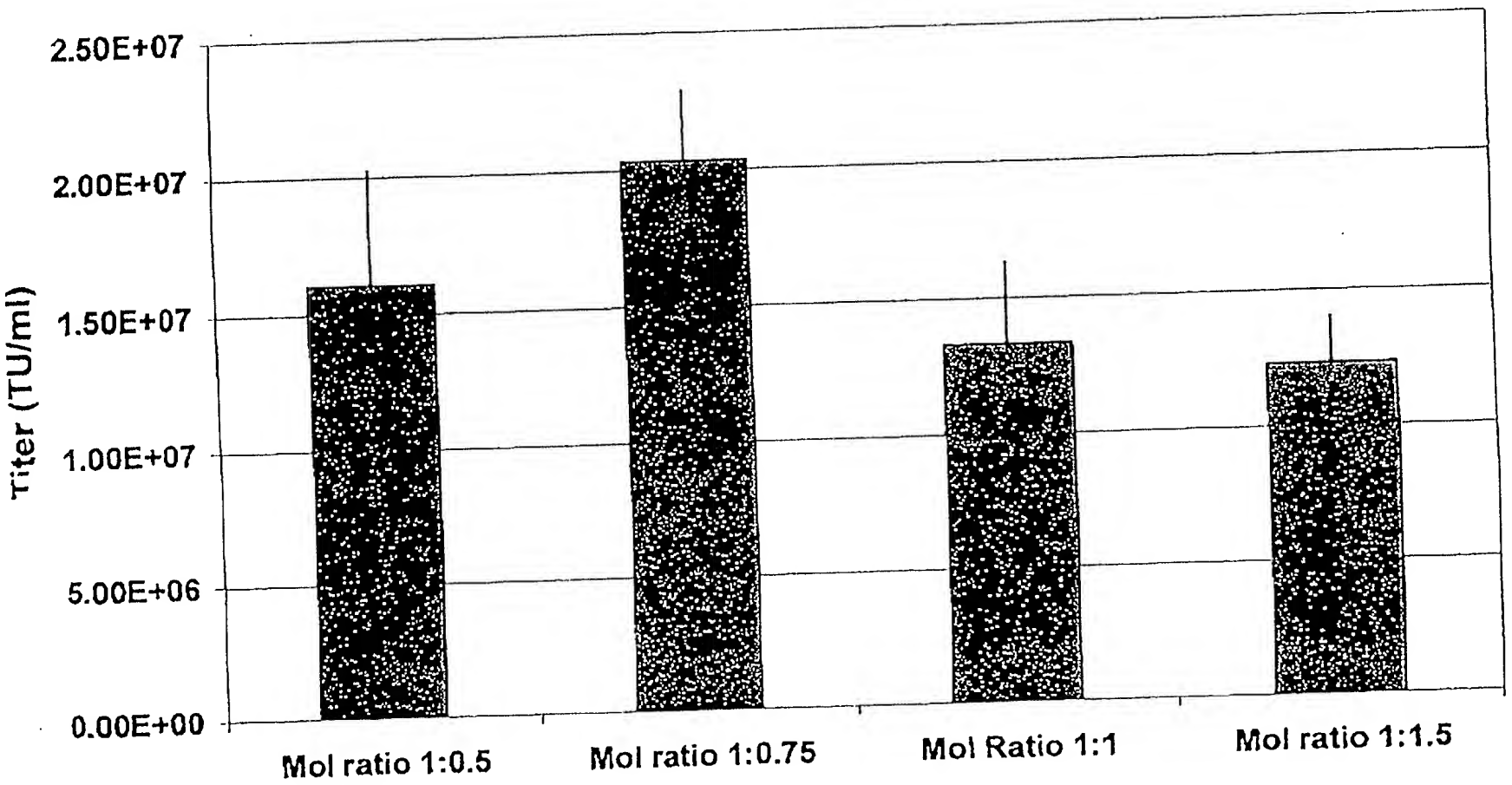


Ratio Optimization for pN1(cPT)GFP Vectors



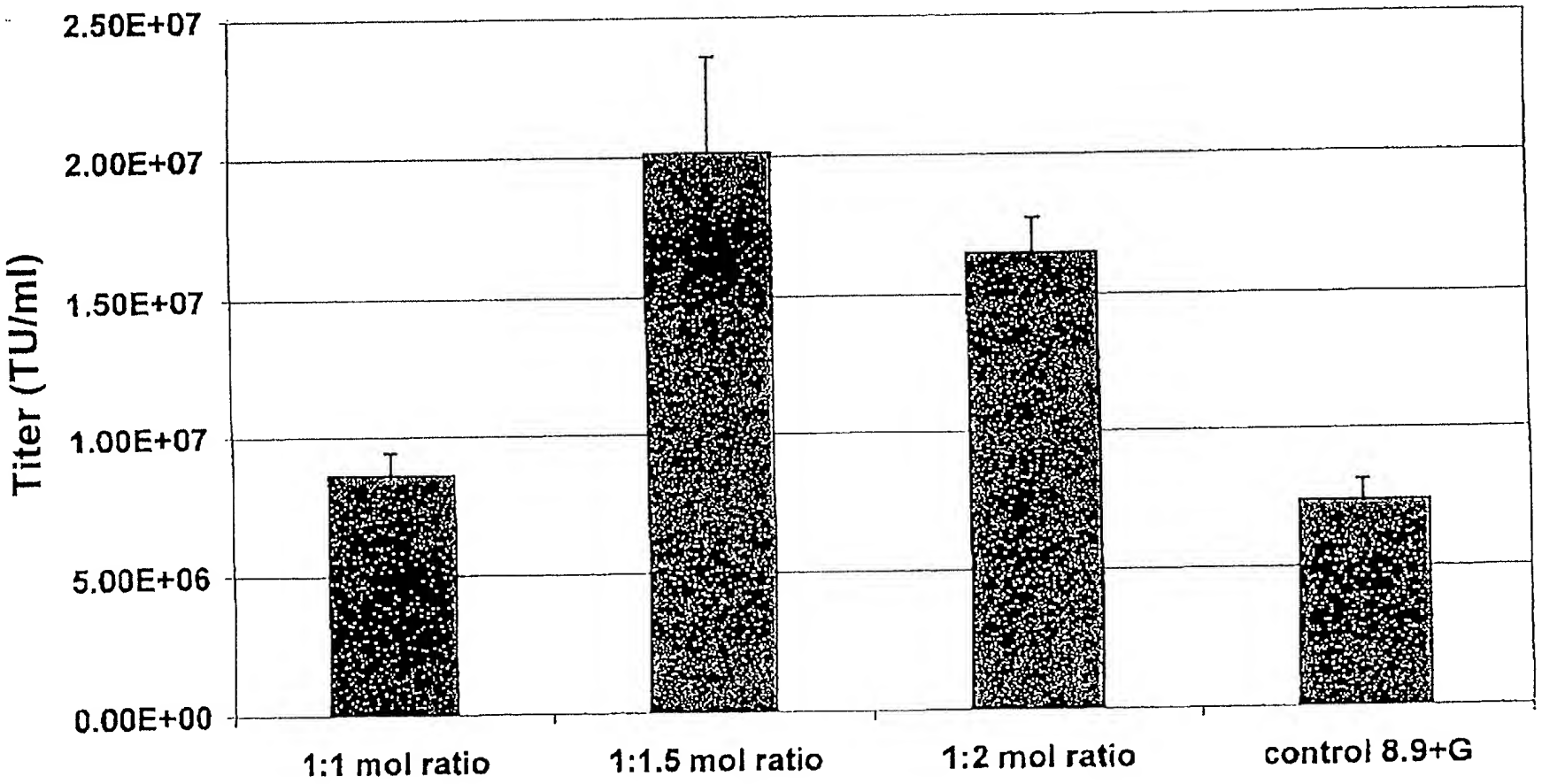
3C

Ratio Optimization for pN1(cPT2)ASenvGFP Vector



3D

Best Vector to Packaging Ratio for pN1cGFP Vector



3E

Optimiztion of vector to packaging ratio for pN2cGFP

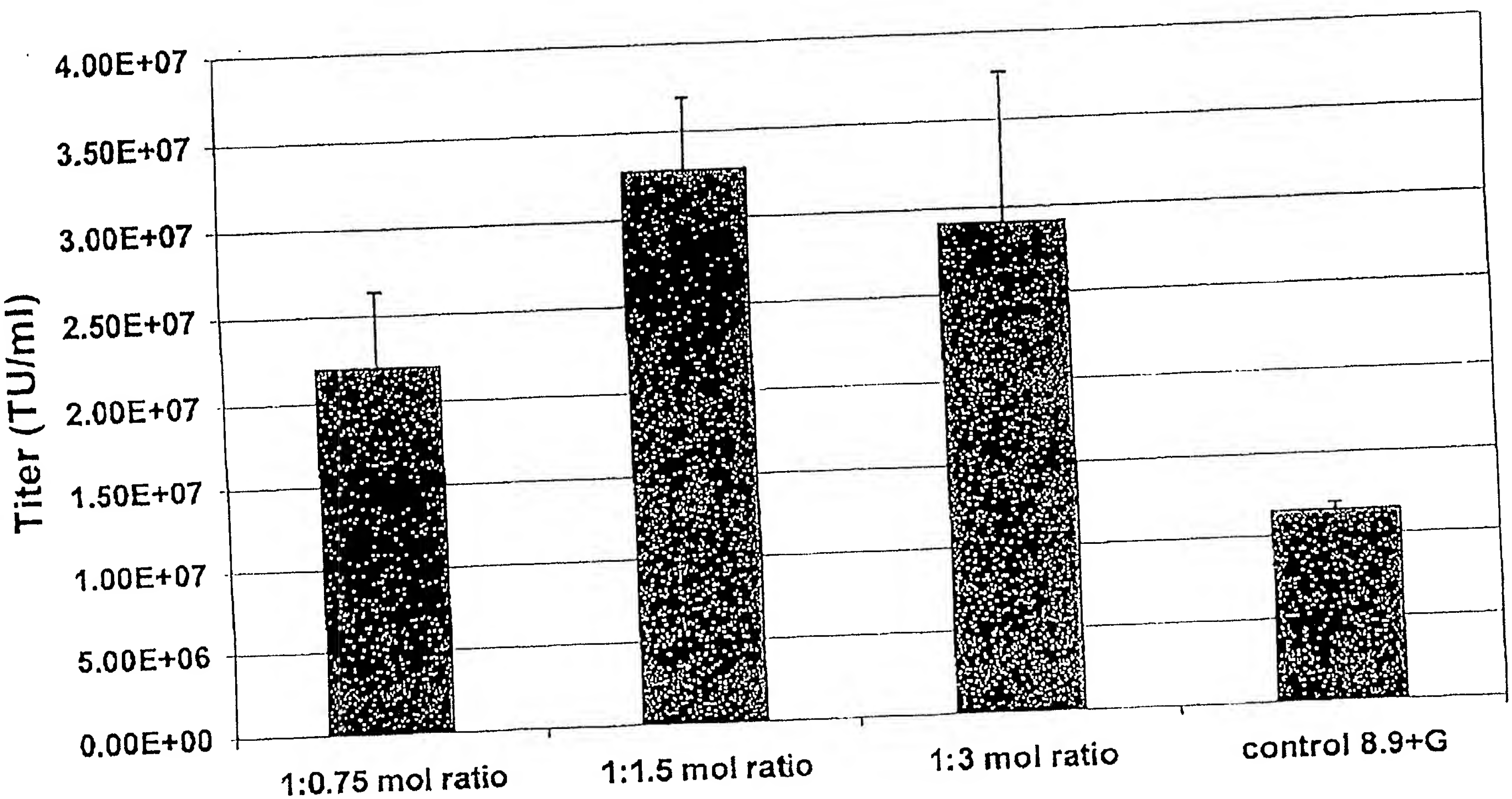


Fig 4A

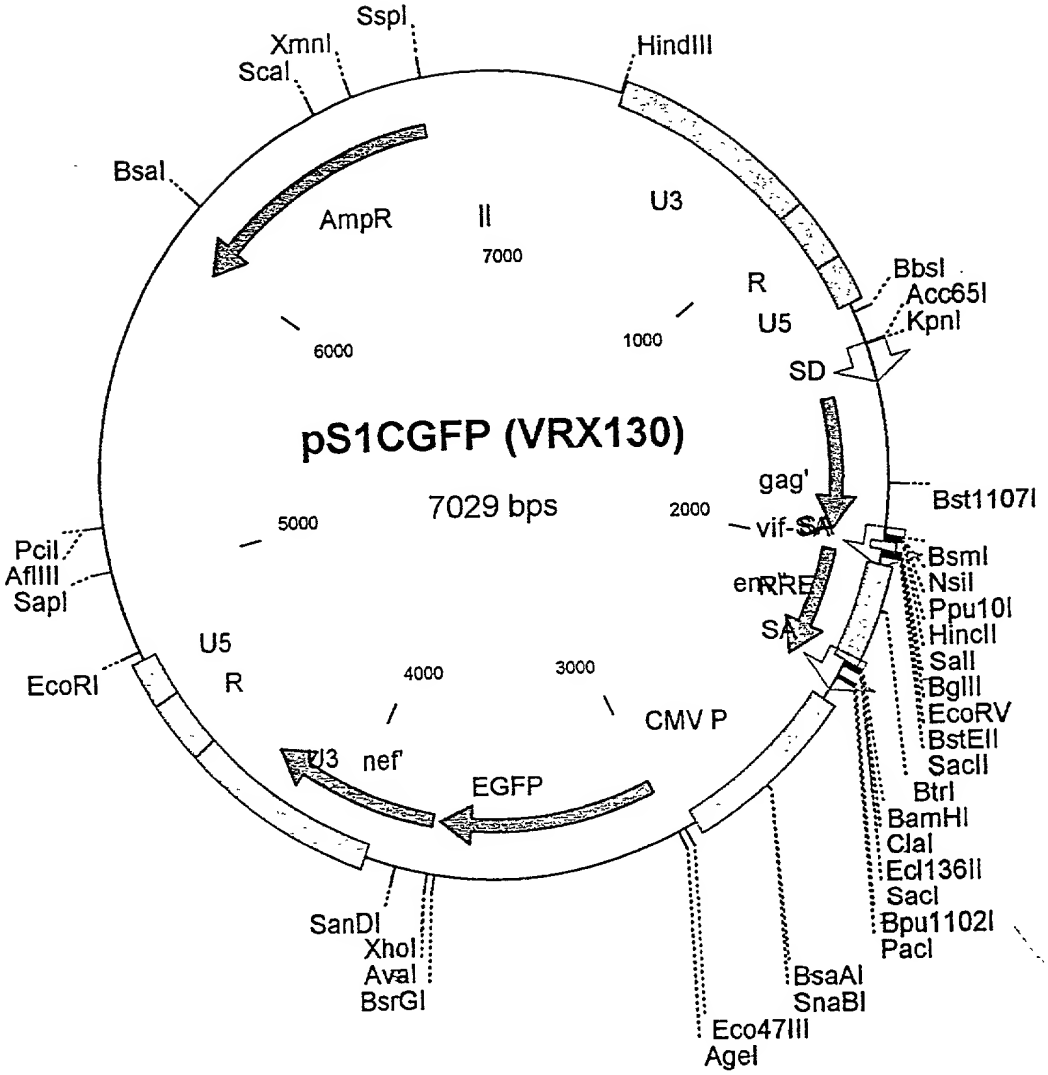
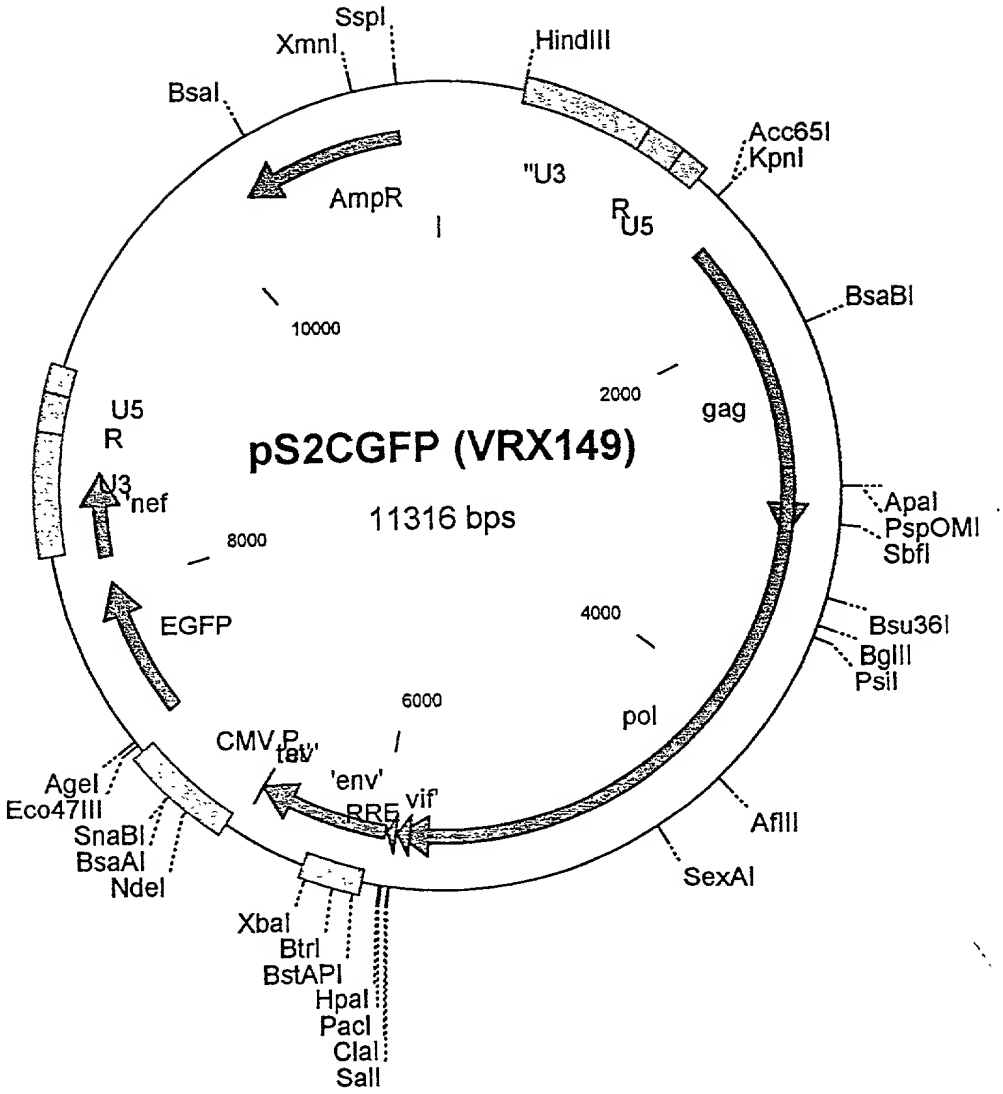
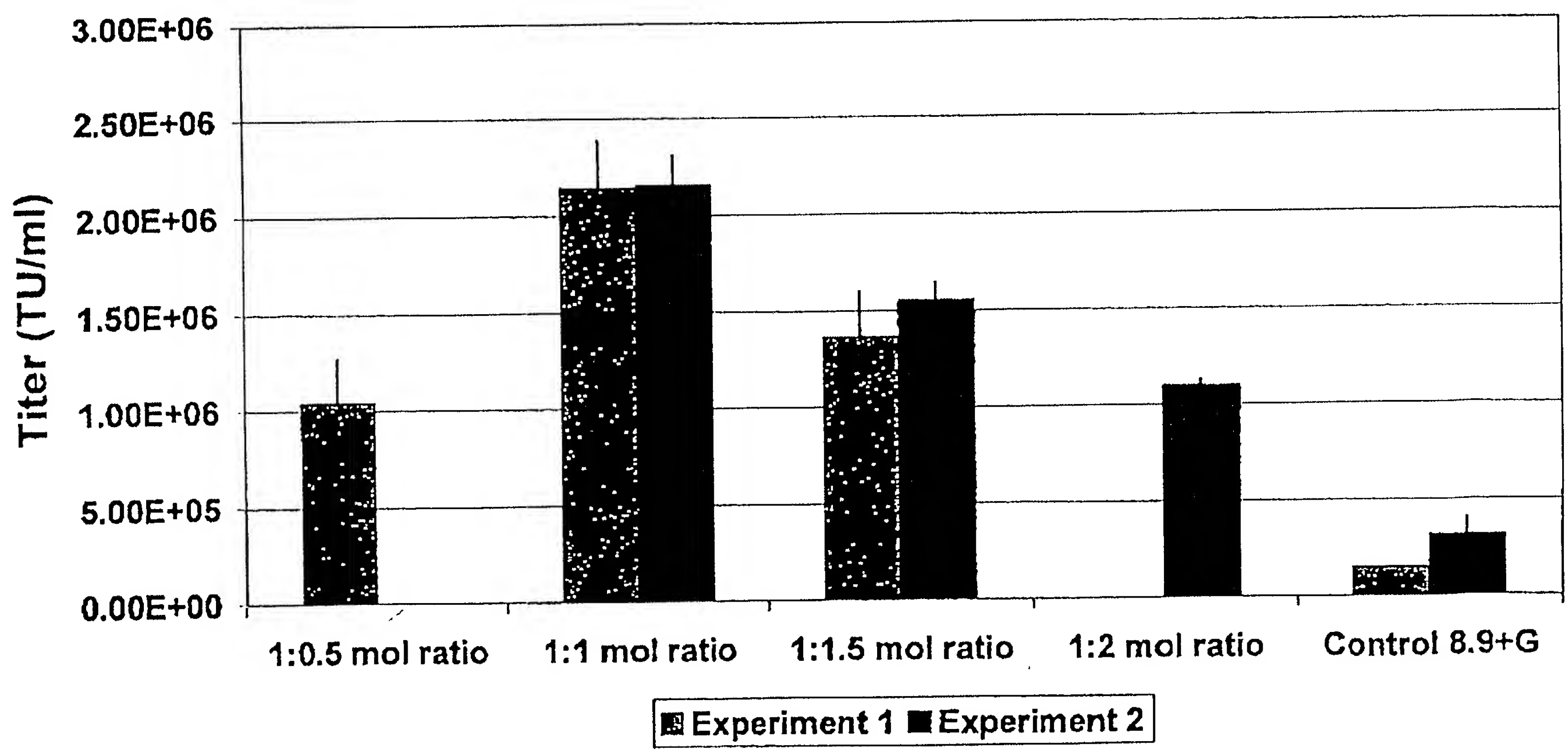

pS1CGFP (VRX130)
7029 bps
SspI
XmnI
ScaI
HindIII
BsaI
AmpR
II
7000
U3
R
U5
1000
2000
6000
5000
BbsI
Acc65I
KpnI
SD
gag'
Bst1107I
vif-SA
BsmI
NsiI
Ppu10I
HincII
SalI
BglII
EcoRV
BstEII
SacII
BtrI
BamHI
ClaI
Ecl136II
SacI
Bpu1102I
PacI
emRRE
SA
CMV P
3000
4000
PciI
AflIII
SapI
EcoRI
U5
R
U3
nef'
EGFP
SanDI
XhoI
AvaI
BsrGI
BsaAI
SnaBI
Eco47III
AgeI

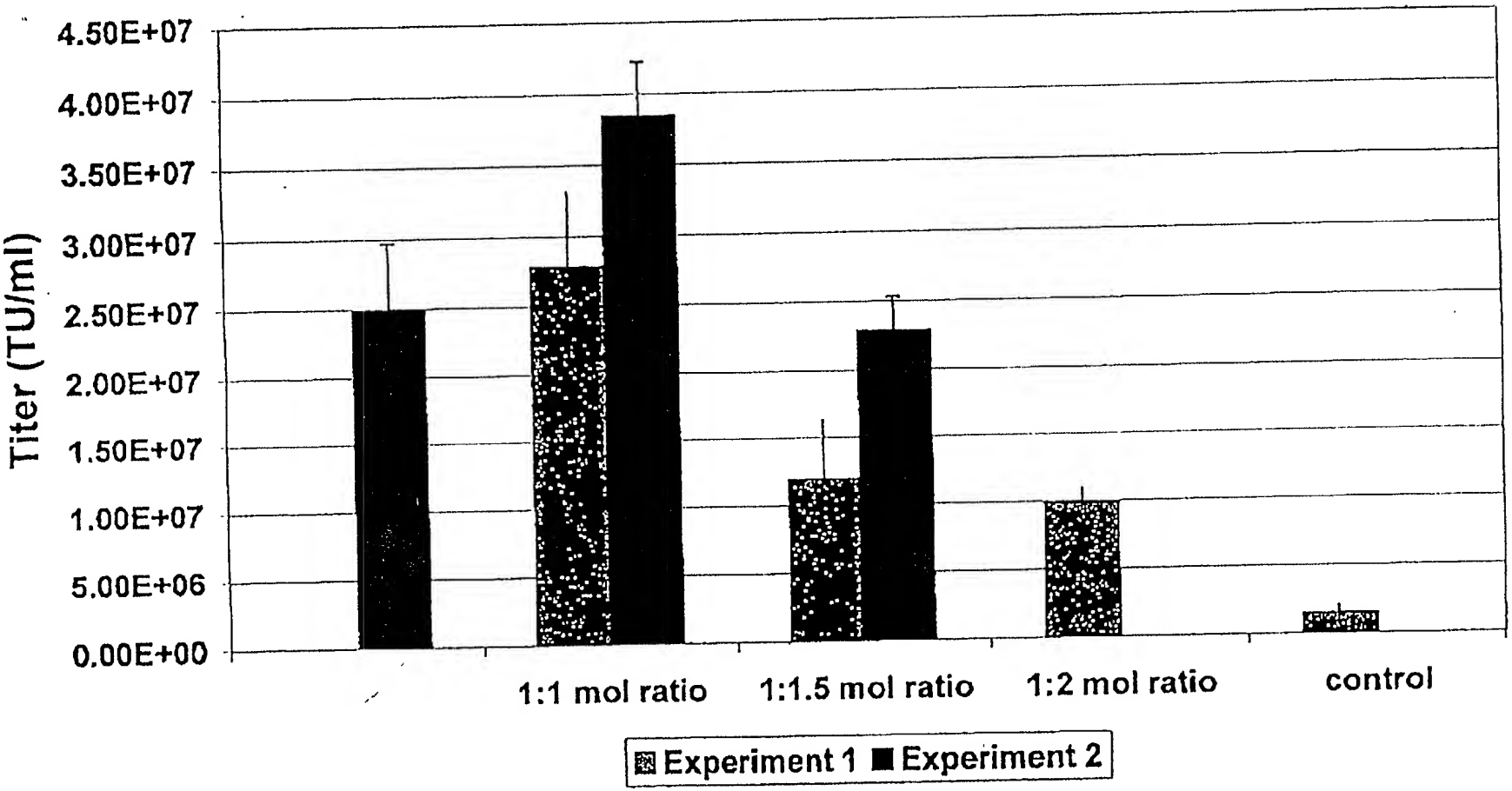
Fig 4B


pS2CGFP (VRX149)
11316 bps
SspI
XmnI
HindIII
BsaI
Acc65I
KpnI
AmpR
"U3
R
U5
BsaBI
10000
2000
gag
U5
R
U3
nef
ApaI
PspOMI
SbfI
8000
4000
EGFP
Bsu36I
BglII
PsiI
6000
pol
CMV P
tat'
'env'
vif'
RRE
AgeI
Eco47III
SnaBI
BsaAI
NdeI
AflII
SexAI
XbaI
BtrI
BstAPI
HpaI
PacI
ClaI
SalI

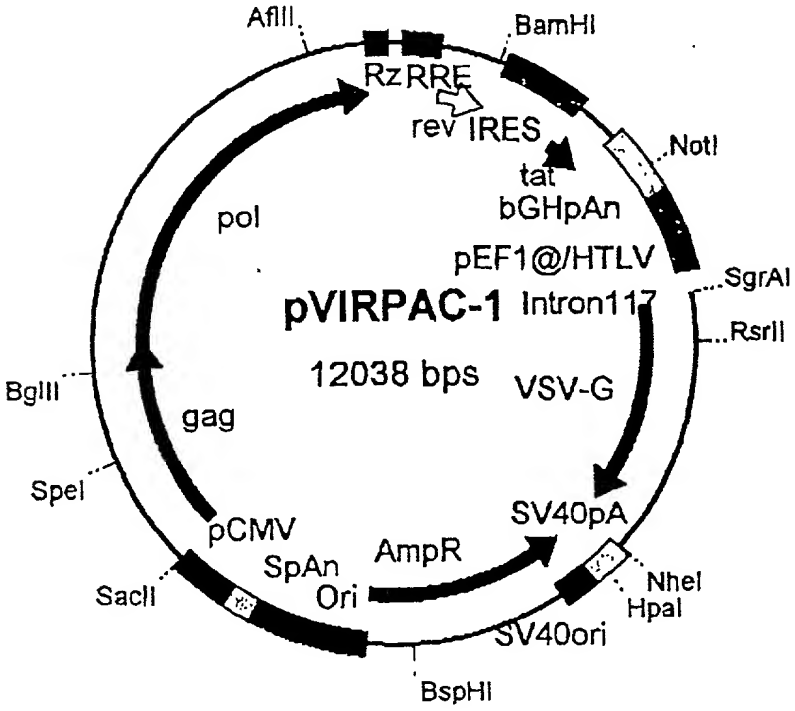
Ratio Optimization for Packaging of pS1cGFP vectors.



Optimiztion of vector to packaging ratio for pS2cGFP



Packaging Construct



New features:

- First 42 nt of gag are degenerated.
- Tat and rev represented as cDNA.
- First 208 nt of rev and last 183 nt of tat are degenerated.
- RRE from HIV-2 is used instead of HIV-1 RRE.

These features eliminate almost any homology with the vector plasmid, make system safer.

- Anti-U5 ribozyme is expressed within gag/pol/RRE cassette, further improving safety.
- Gag/pol/rev/tat/RRE cassette and VSV-G expressed from the same plasmid. This feature may enhance packaging efficiency and titers of the vectors.

Fig. 6B Packaging Plasmid for Second Generation Vectors

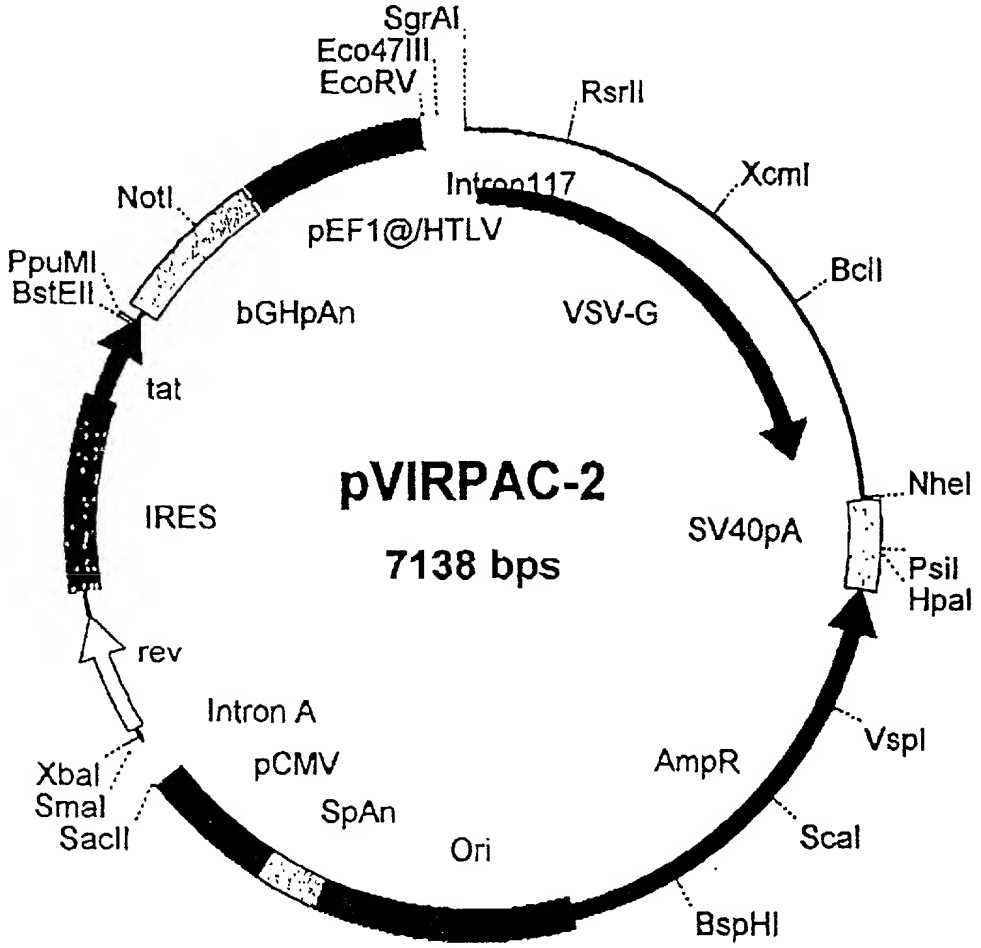


Fig. 6C Packaging Plasmid for First Generation Vectors

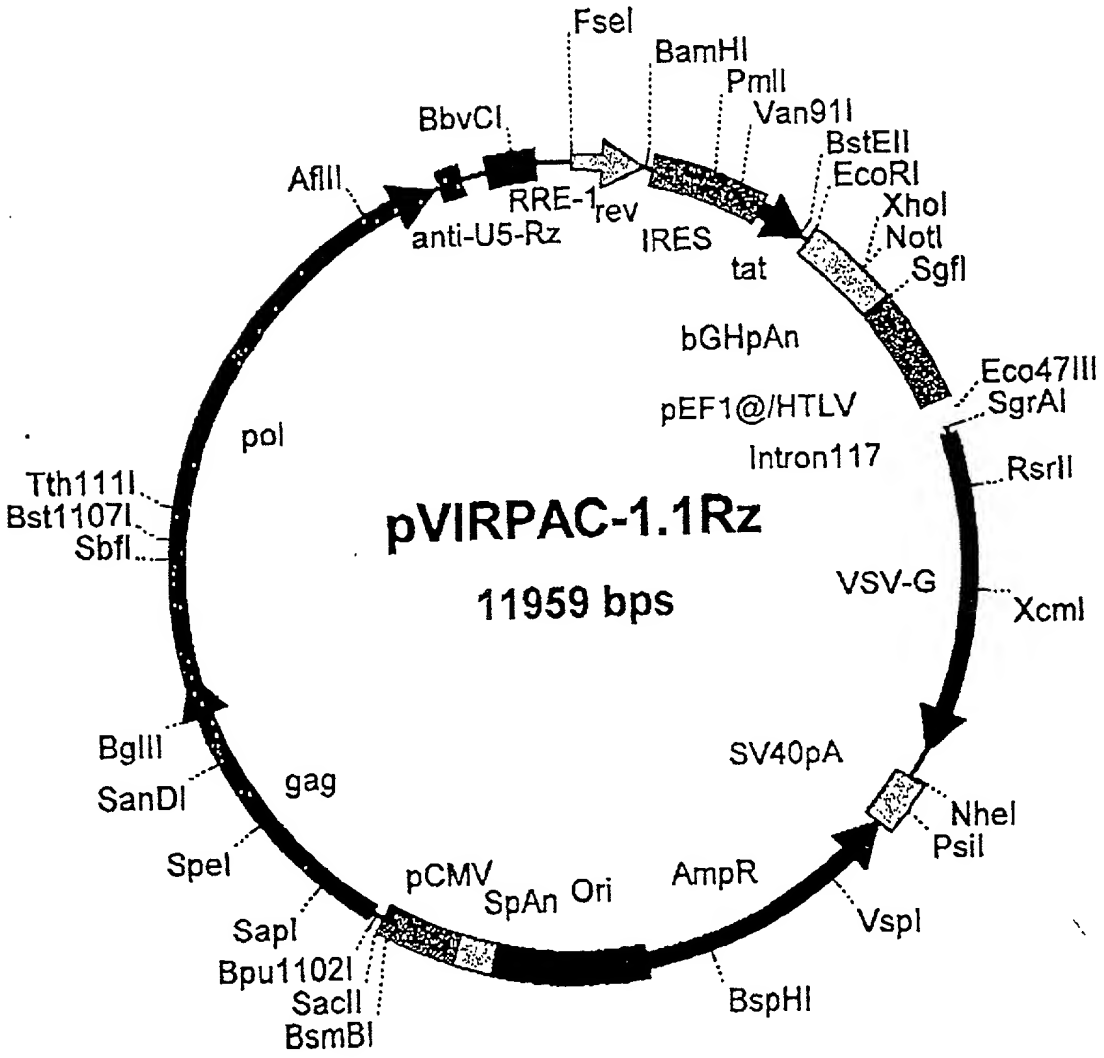


Fig 6D

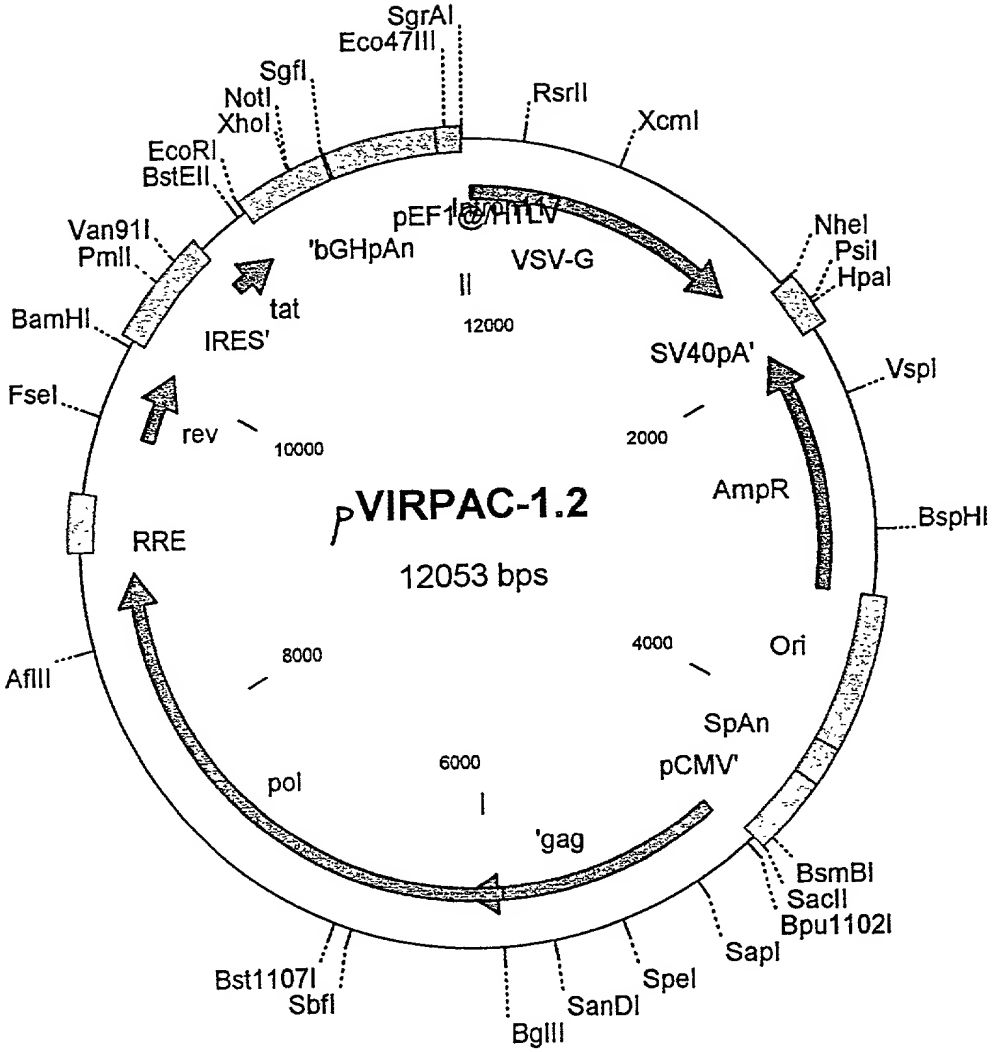

SgrAI
Eco47III
SgfI
NotI
XhoI
EcoRI
BstEII
Van91I
PmlI
BamHI
FseI
AflII
RsrII
XcmI
NheI
PsiI
HpaI
VspI
BspHI
BsmBI
SacII
Bpu1102I
SapI
SpeI
SanDI
BglII
Bst1107I
SbfI
pEF1α/HTLV
'bGHpAn
VSV-G
tat
IRES'
rev
SV40pA'
AmpR
Ori
SpAn
pCMV'
'gag
pol
RRE
pVIRPAC-1.2
12053 bps
2000
4000
6000
8000
10000
12000

Fig 6E

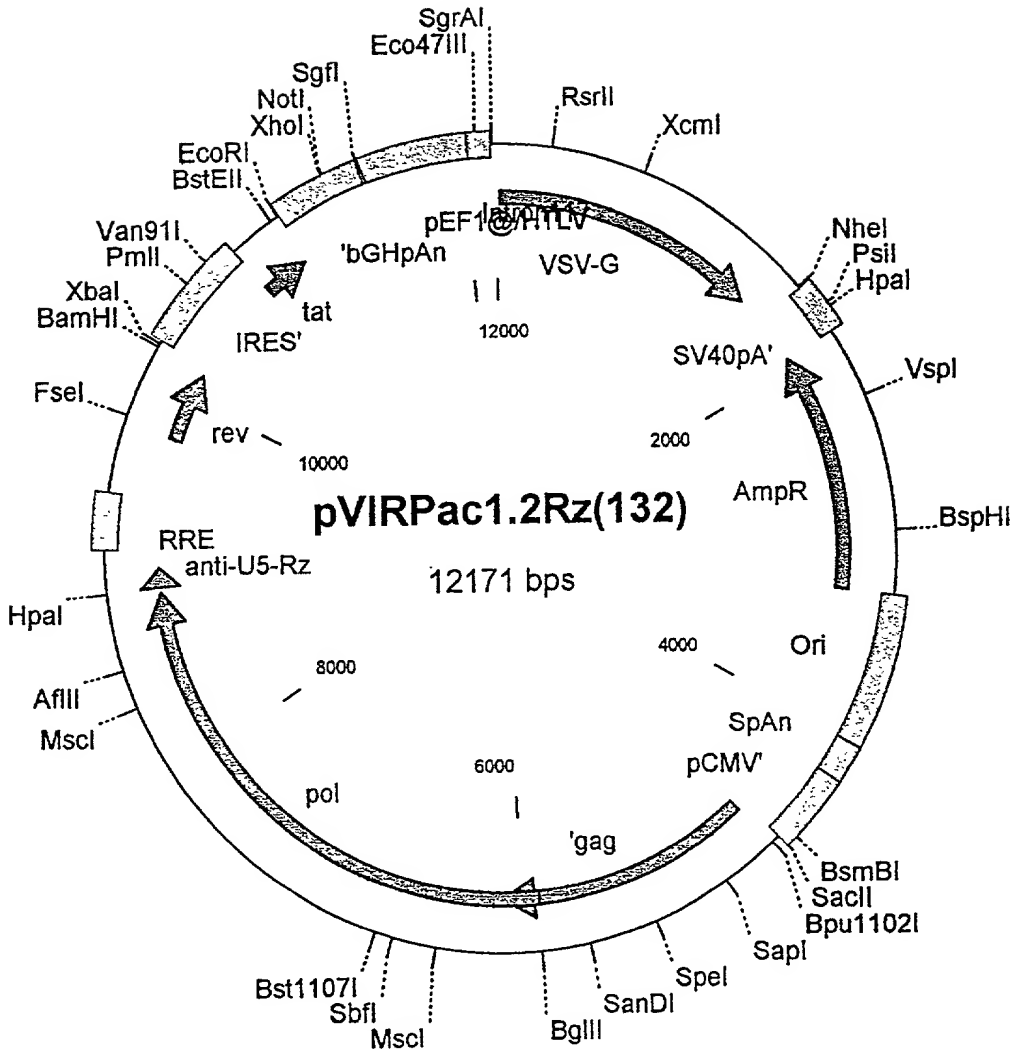

pVIRPac1.2Rz(132)
12171 bps
SgrAI
Eco47III
SgfI
NotI
XhoI
EcoRI
BstEII
Van91I
PmlI
XbaI
BamHI
FseI
HpaI
AflII
MscI
Bst1107I
SbfI
MscI
BglII
SanDI
SpeI
SapI
BsmBI
SacII
Bpu1102I
BspHI
VspI
NheI
PsiI
HpaI
RsrII
XcmI
pEF1α/HTLV
'bGHpAn
VSV-G
tat
IRES'
rev
RRE
anti-U5-Rz
pol
'gag
pCMV'
SpAn
Ori
AmpR
SV40pA'
2000
4000
6000
8000
10000
12000

Fig 6F

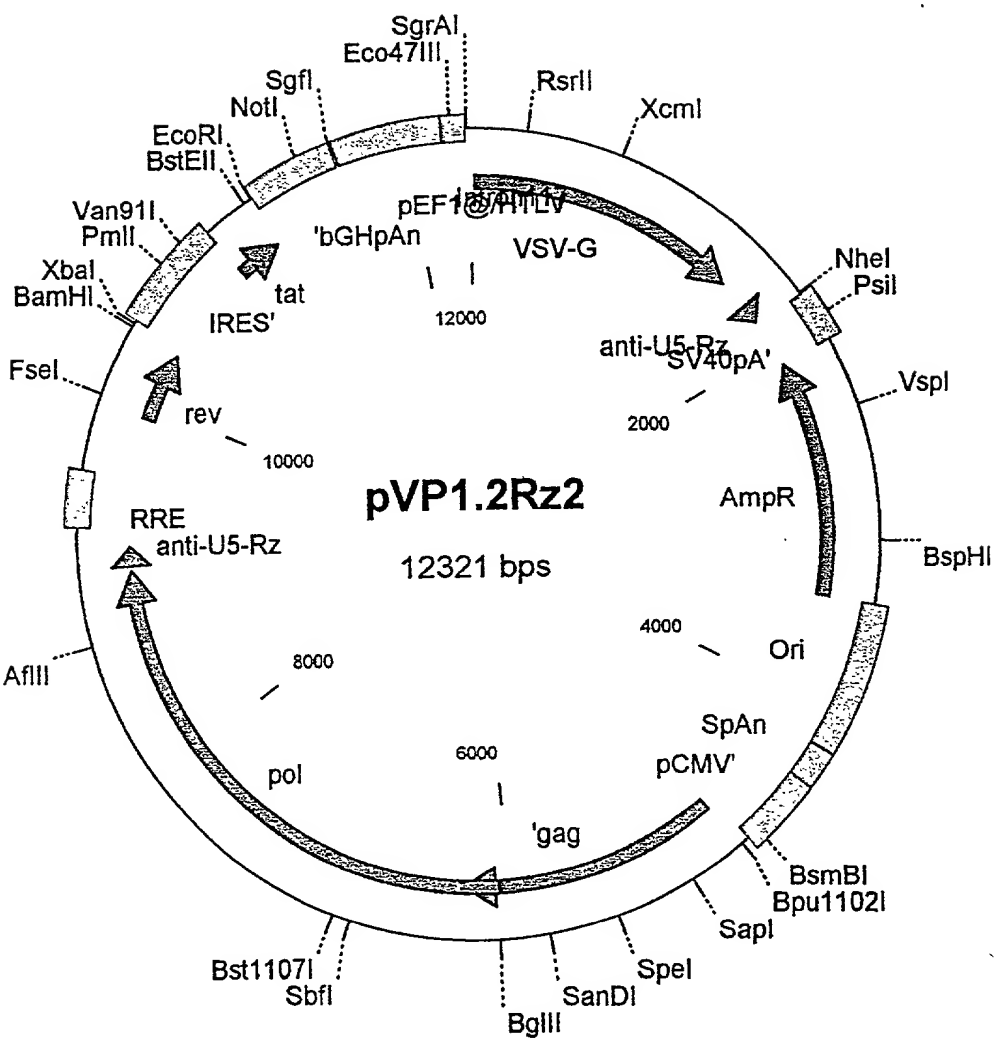

SgrAI
Eco47III
SgfI
NotI
EcoRI
BstEII
Van91I
PmlI
XbaI
BamHI
FseI
RRE
anti-U5-Rz
AflII
Bst1107I
SbfI
BglII
SanDI
SpeI
SapI
BsmBI
Bpu1102I
BspHI
VspI
NheI
PsiI
RsrII
XcmI
pEF1α/HTLV
VSV-G
'bGHpAn
tat
IRES'
rev
anti-U5-Rz
SV40pA'
AmpR
Ori
SpAn
pCMV'
'gag
pol
12000
2000
4000
6000
8000
10000
pVP1.2Rz2
12321 bps

Fig 6G

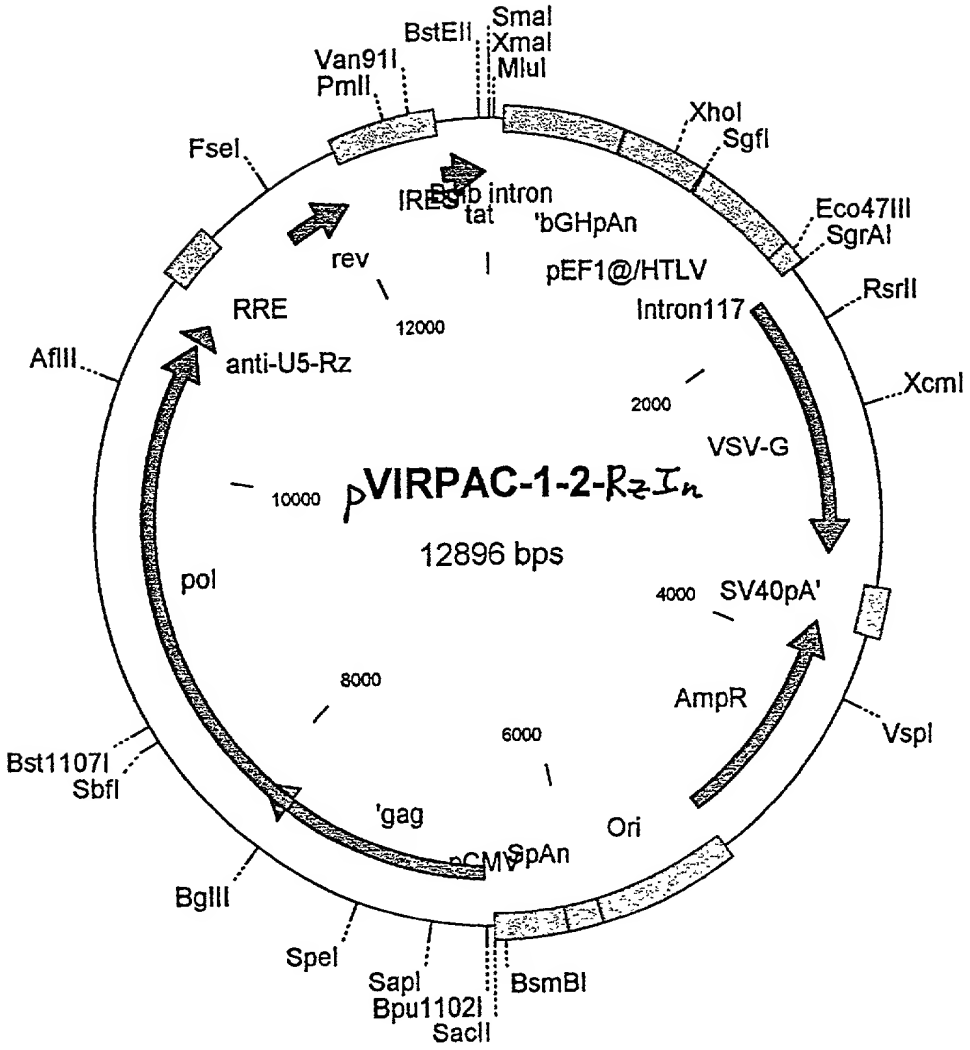
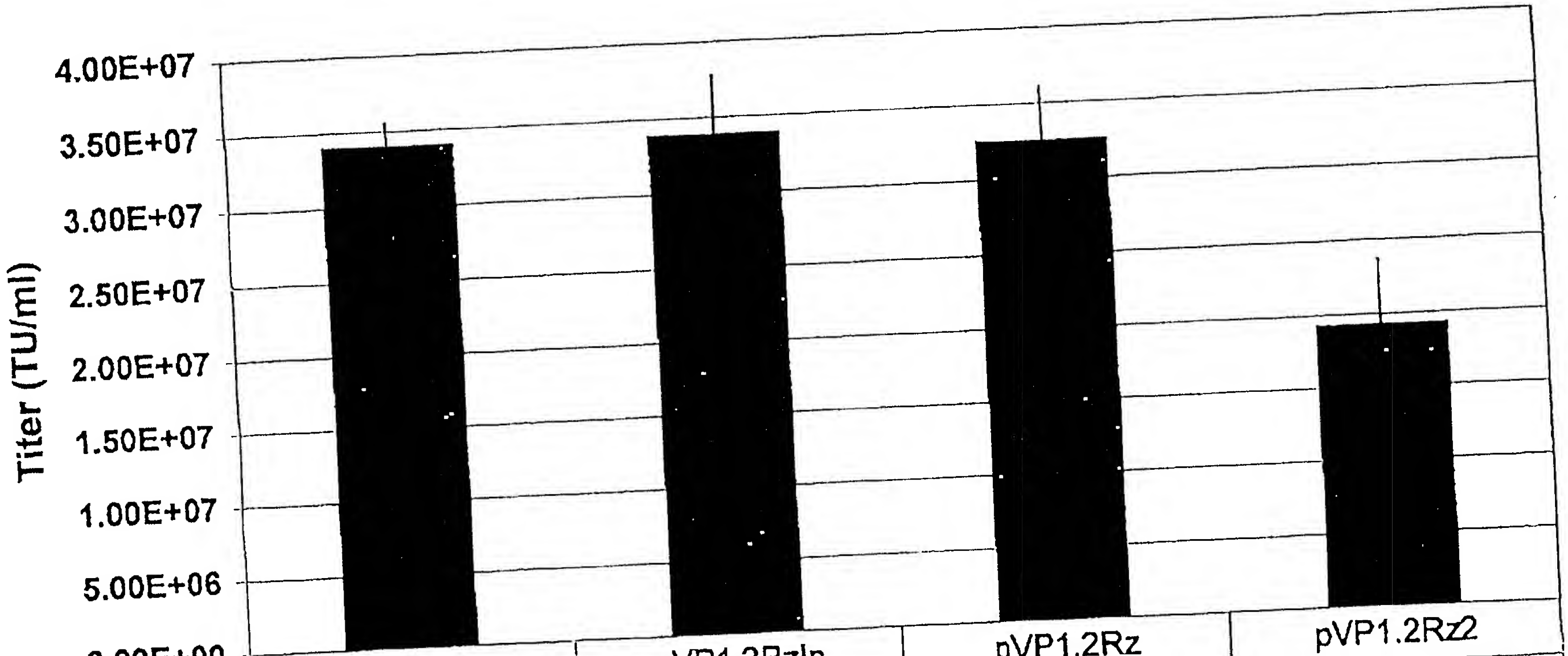

pVIRPAC-1-2-RzIn
12896 bps
SmaI
XmaI
MluI
BstEII
Van91I
PmlI
FseI
XhoI
SgfI
Eco47III
SgrAI
RsrII
XcmI
VsplI
AmpR
Ori
SV40pA'
VSV-G
Intron117
pEF1@/HTLV
'bGHpAn
tat
rev
RRE
anti-U5-Rz
AflII
pol
'gag
Bst1107I
SbfI
BglII
SpeI
SapI
Bpu1102I
SacII
BsmBI
2000
4000
6000
8000
10000
12000

Fig 7

Influence of Ribozyme(s) in the Packaging on pN1(cPT)GFP Vector Titers in HeLa-tat Cells



	pVP1.2	pVP1.2RzIn	pVP1.2Rz	pVP1.2Rz2
Average	3.39E+07	3.38E+07	3.25E+07	1.92E+07

Fig 8

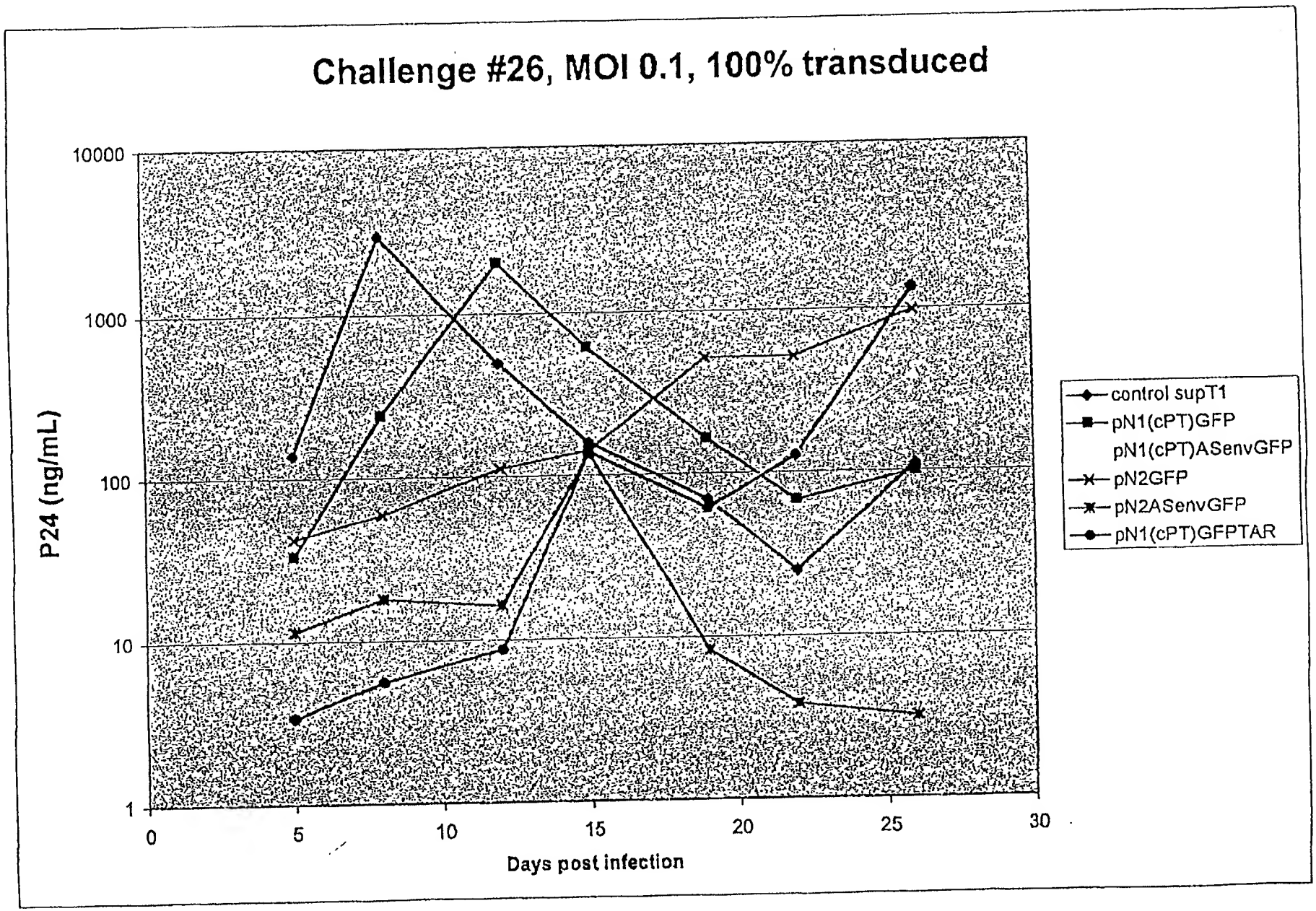

Challenge #26, MOI 0.1, 100% transduced
P24 (ng/mL)
10000
1000
100
10
1
0
5
10
15
20
25
30
Days post infection
control supT1
pN1(cPT)GFP
pN1(cPT)ASenvGFP
pN2GFP
pN2ASenvGFP
pN1(cPT)GFPTAR

FIGURE 9A

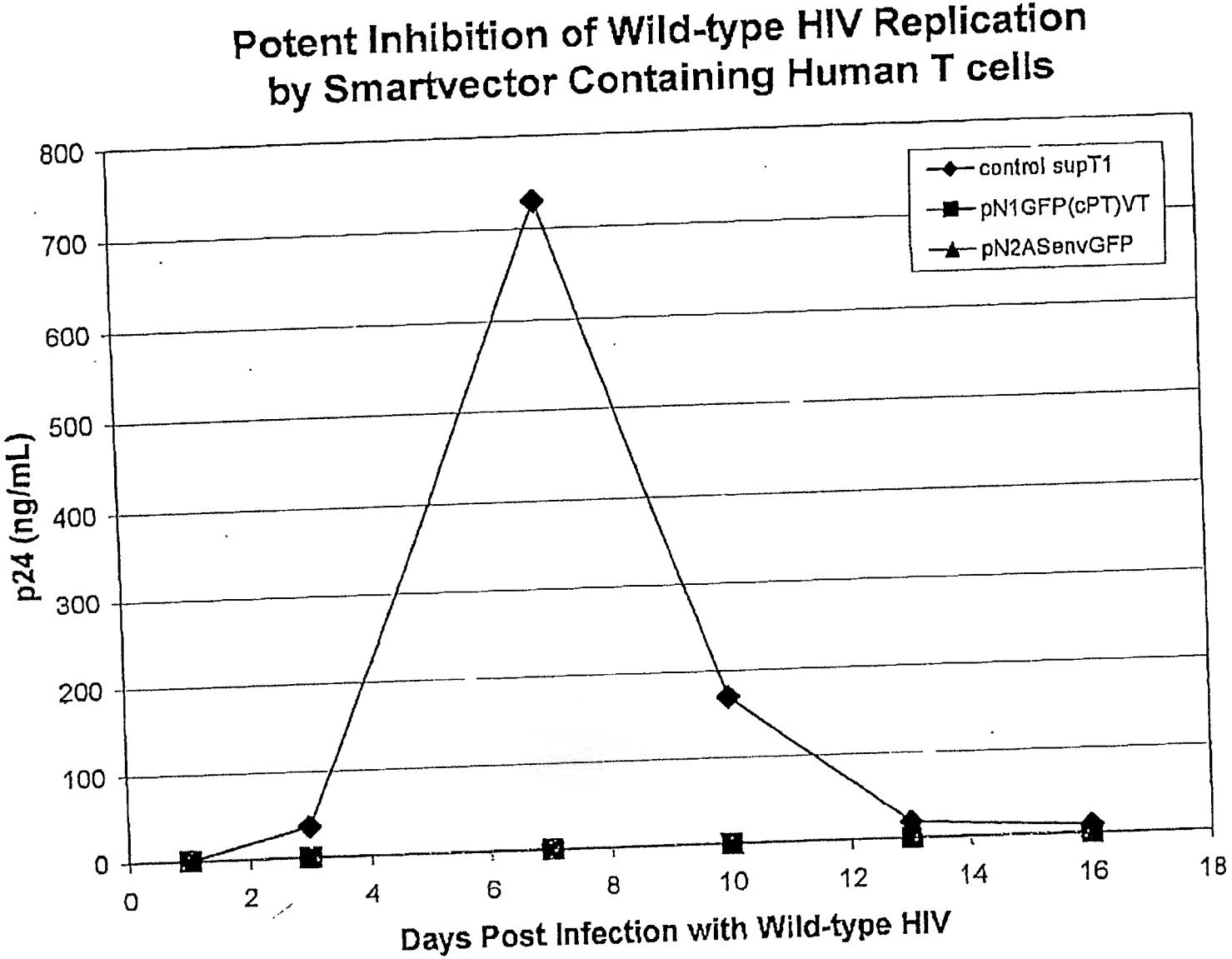


FIGURE 9B

Potent Inhibition of Wild-type HIV Replication by Smartvector Containing T Cells

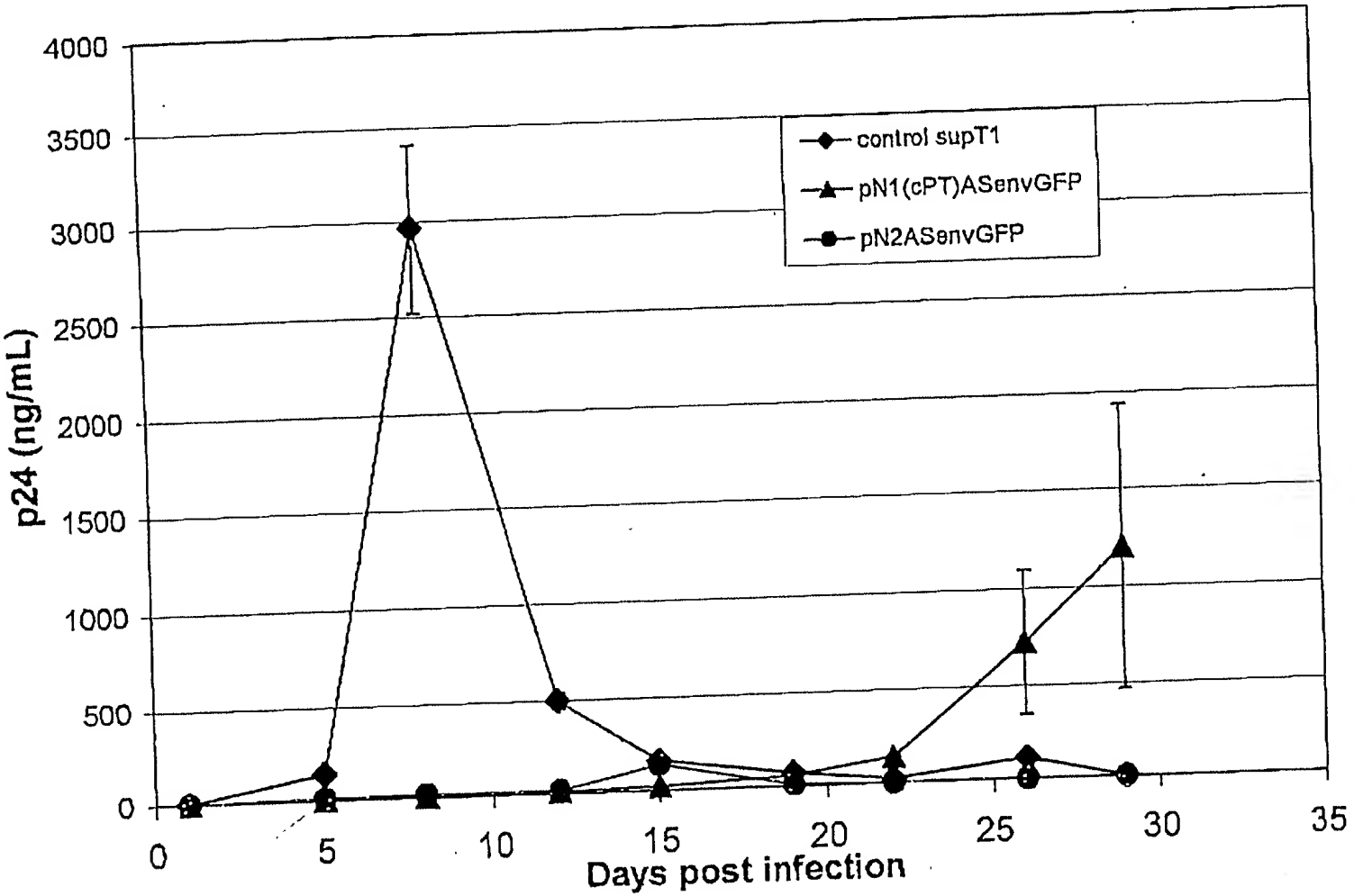
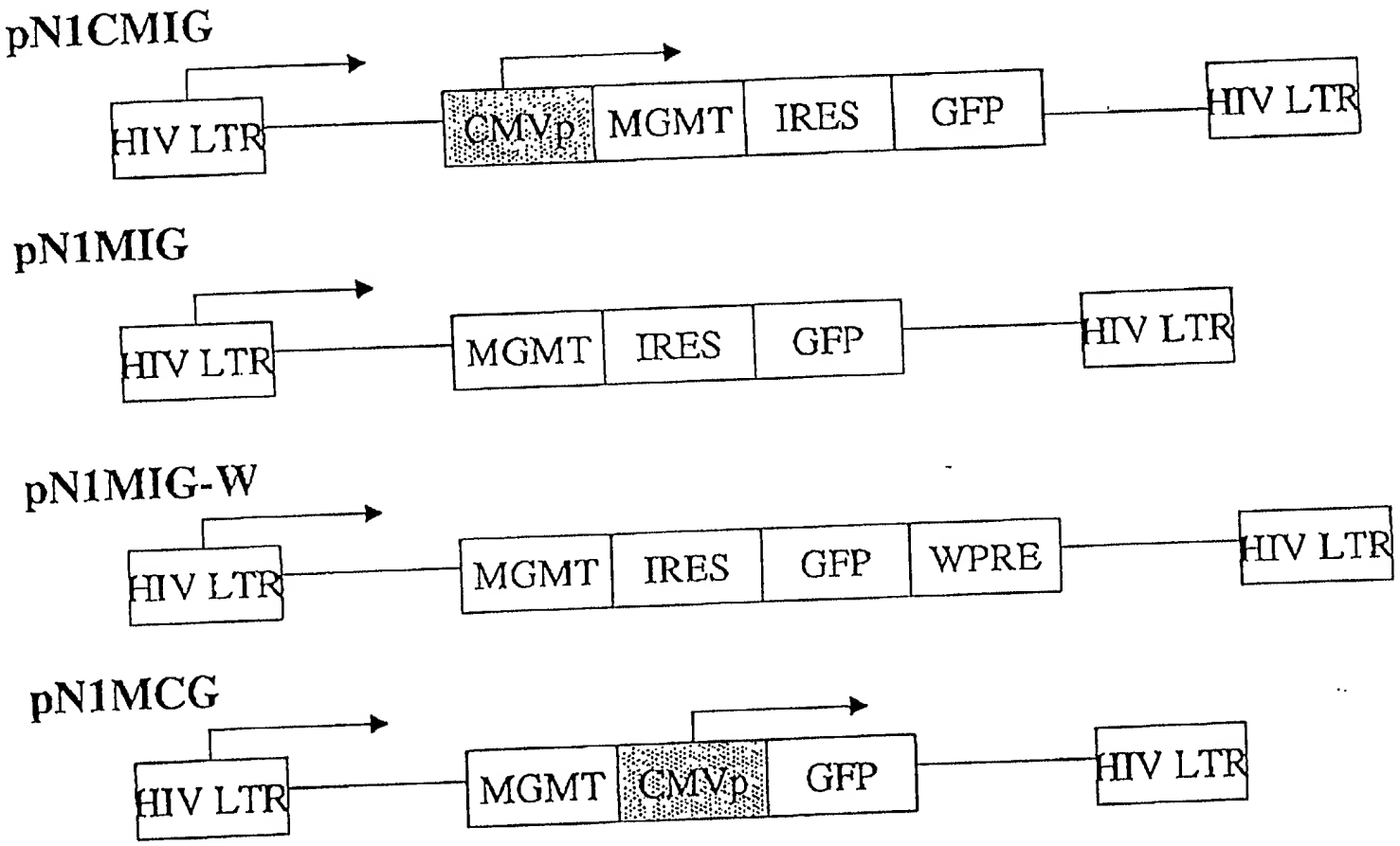


Fig 10A



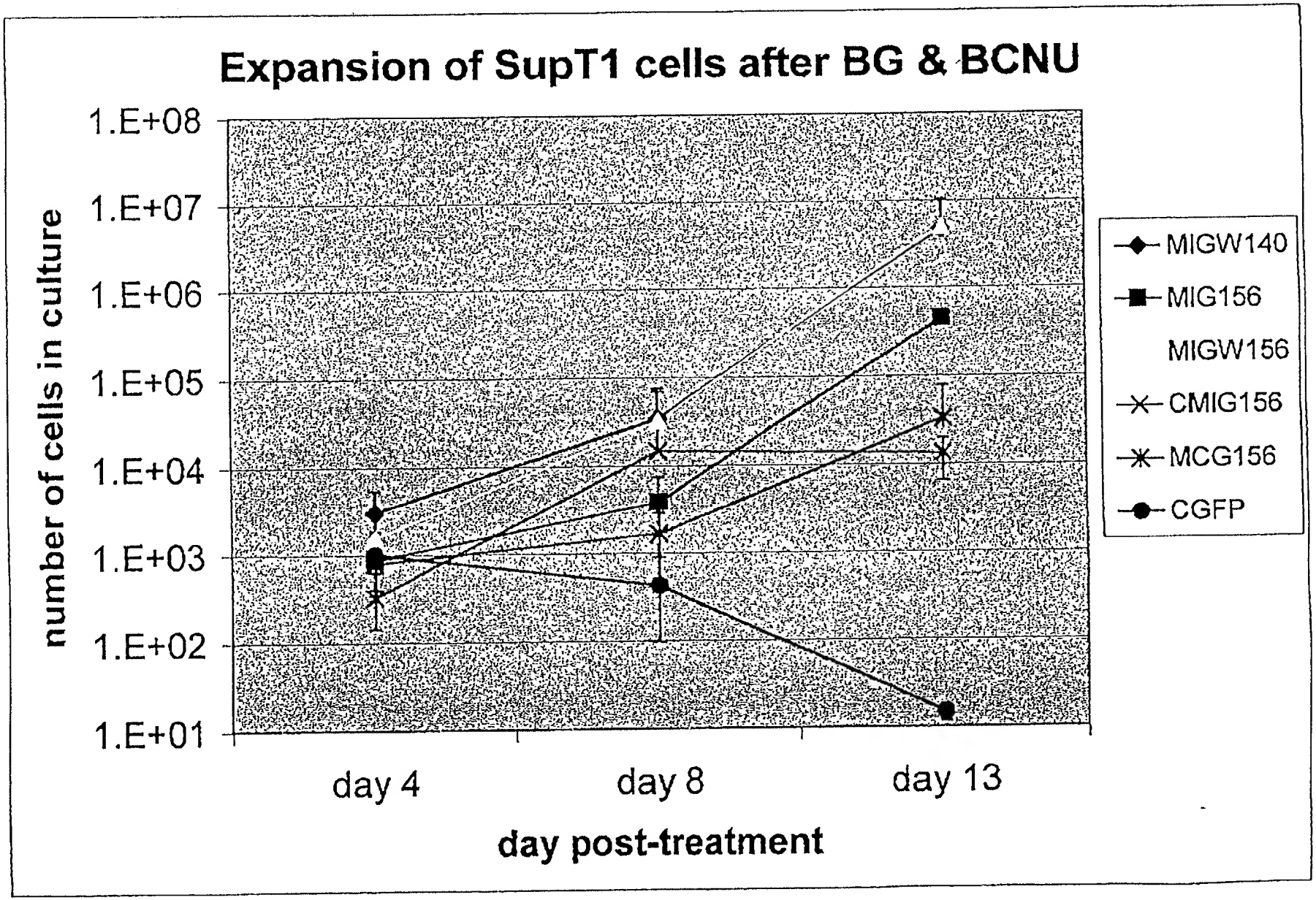
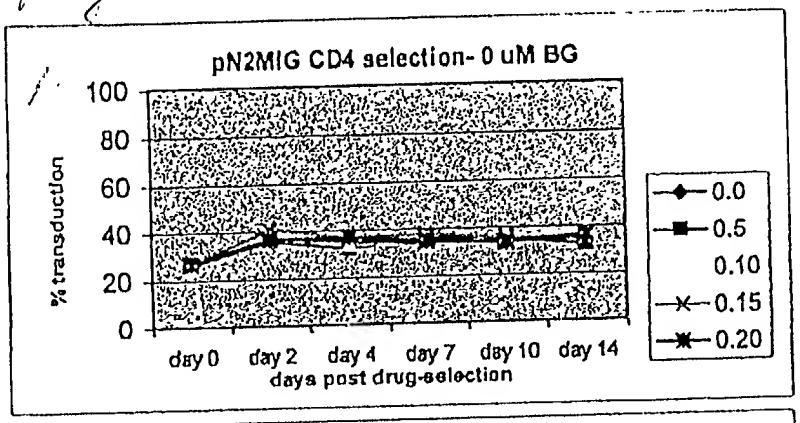
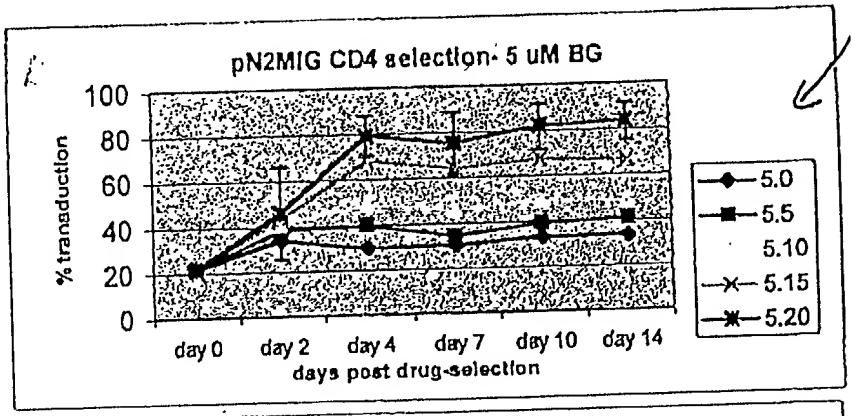
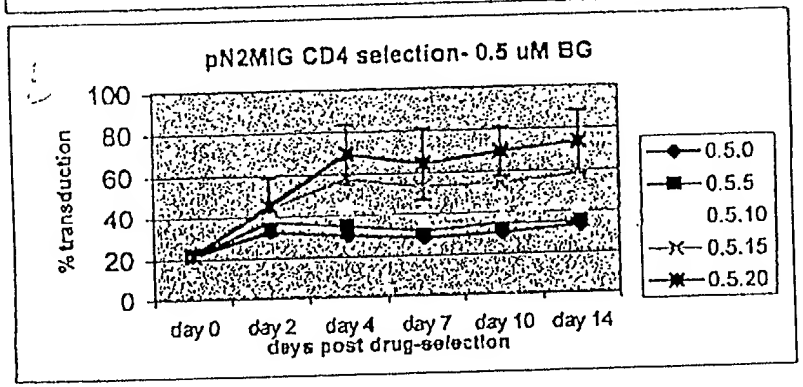
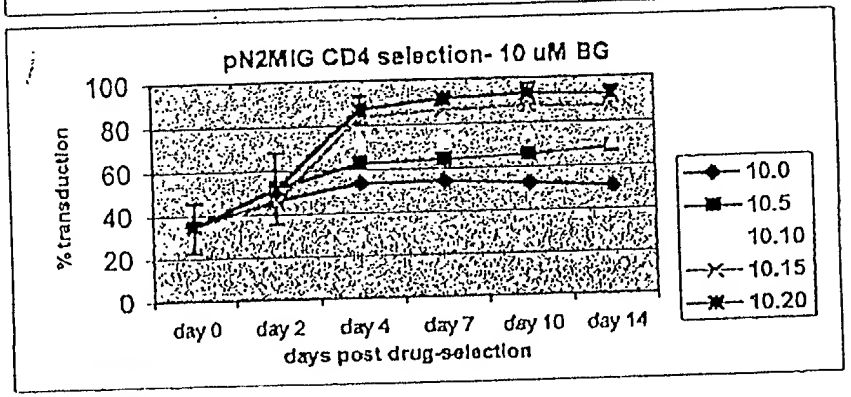

Expansion of SupT1 cells after BG & BCNU
number of cells in culture
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
day 4
day 8
day 13
day post-treatment
MIGW140
MIG156
MIGW156
CMIG156
MCG156
CGFP

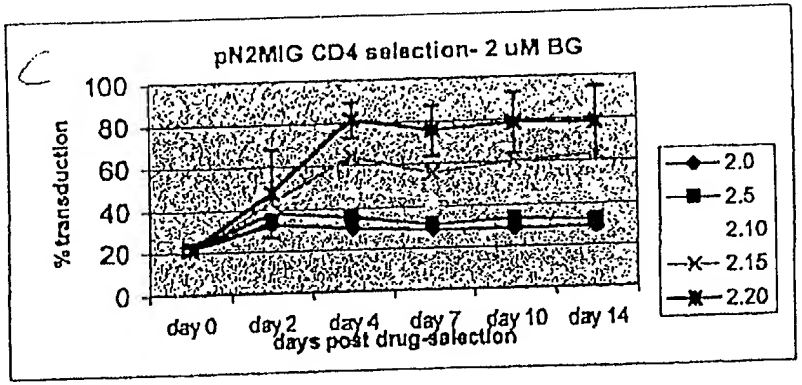
Fig 11

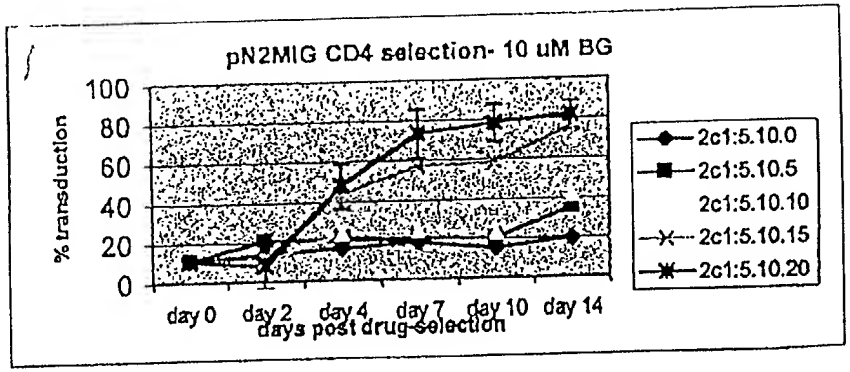

pN2MIG CD4 selection- 0 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
0.0
0.5
0.10
0.15
0.20

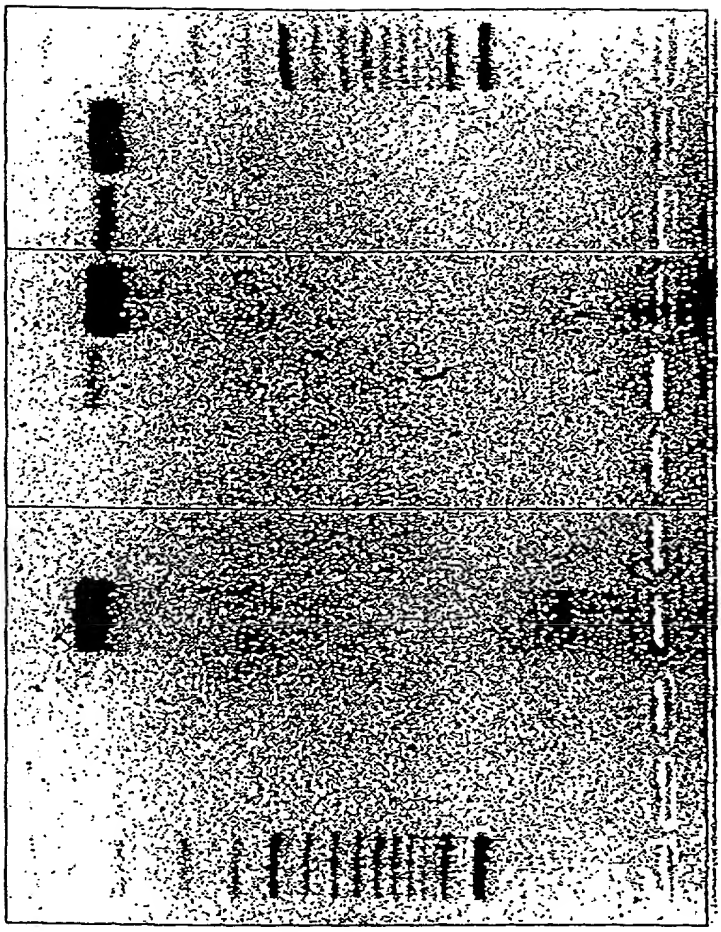

pN2MIG CD4 selection- 5 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
5.0
5.5
5.10
5.15
5.20


pN2MIG CD4 selection- 0.5 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
0.5.0
0.5.5
0.5.10
0.5.15
0.5.20


pN2MIG CD4 selection- 10 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
10.0
10.5
10.10
10.15
10.20


pN2MIG CD4 selection- 2 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
2.0
2.5
2.10
2.15
2.20


pN2MIG CD4 selection- 10 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
2c1:5.10.0
2c1:5.10.5
2c1:5.10.10
2c1:5.10.15
2c1:5.10.20



Marker

1 pN1 CGFP 1C exp 30

3 pN1 CGFP 2C exp 30

1-4 pVP1.2

9-12 pVP1.2 Rz

13-16 pVP1.2 Rz2

pNL4-3 with DNase I

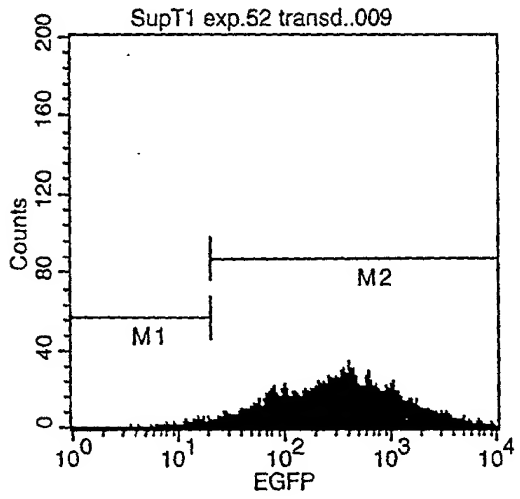
pNL4-3 without DNase I

Amp. Neg. Control

Extraction Neg. Control

Marker

Fig 13A



Histogram Statistics

File: SupT1 exp.52 transd..009 Sample ID: SupT1 e›
Tube: pN1(cPT)ASenvGFP 452 a Acquisition Date: 25-

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	6356	100.00	63.56	570.39
M1	1, 20	95	1.49	0.95	13.86
M2	20, 9910	6262	98.52	62.62	578.74

Fig 12

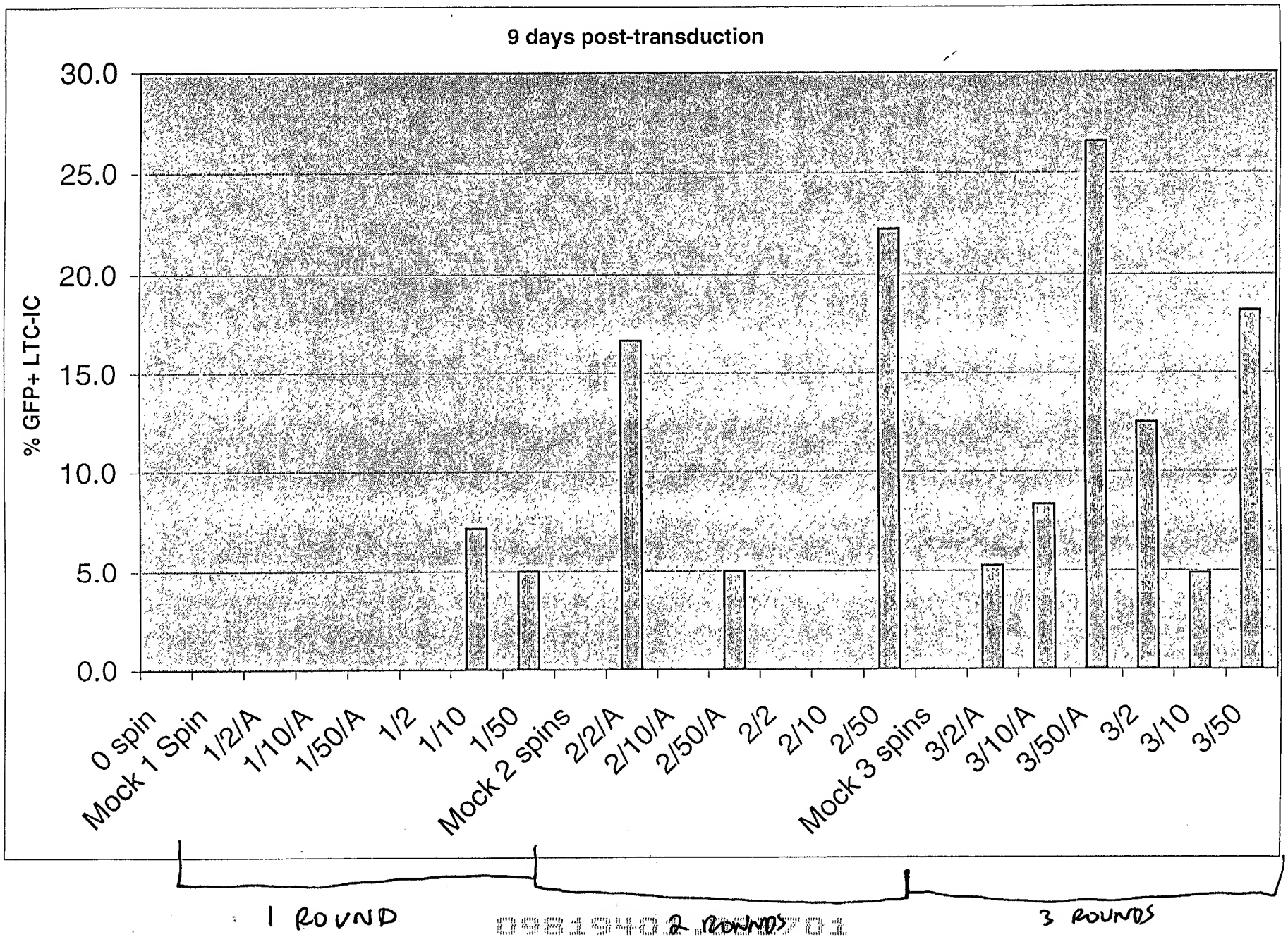

9 days post-transduction
% GFP+ LTC-IC
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0 spin
Mock 1 Spin
1/2/A
1/10/A
1/50/A
1/2
1/10
1/50
Mock 2 spins
2/2/A
2/10/A
2/50/A
2/2
2/10
2/50
Mock 3 spins
3/2/A
3/10/A
3/50/A
3/2
3/10
3/50
1 ROUND
2 ROUNDS
3 ROUNDS

Fig 14A

Vsv-G, RD114 AND RD114-VSV-G CHIMERIA ENVELOPE PROTEINS

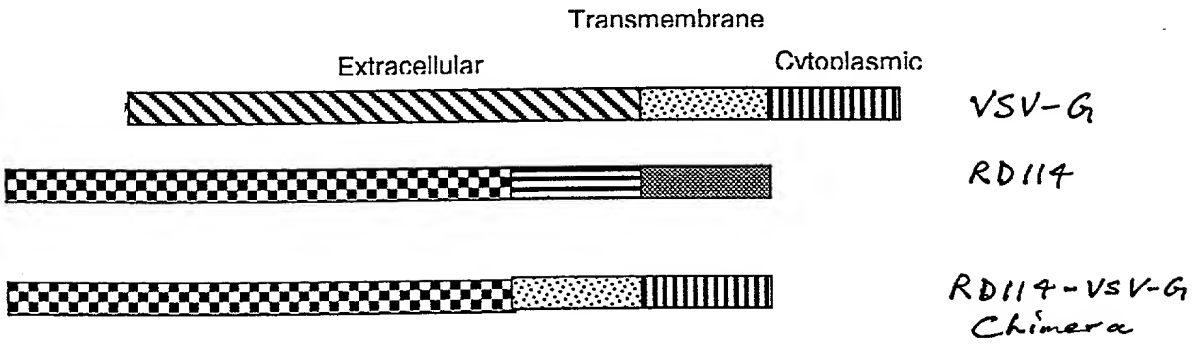


Fig 14B

Titers of RD114-pseudotyped HIV-1 vectors in HT1080

Envelopes	IU/ml
VSV G	3.5x10e6
Rabies virus G	1.6x10e6
RD114WT env	1.5x10e5
RD114E env	3.8x10e4

Fig 15A

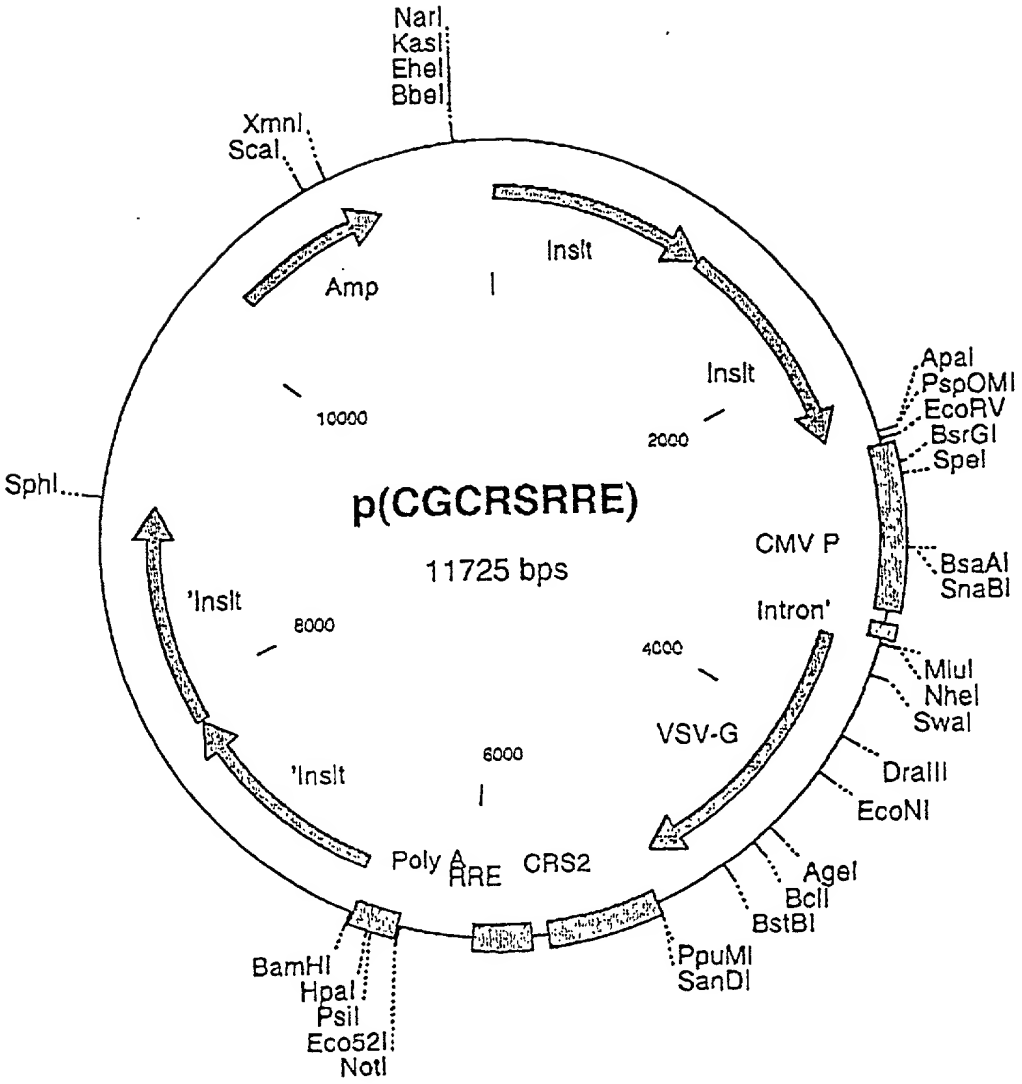

NarI
KasI
EheI
BbeI
XmnI
ScaI
InsIt
Amp
InsIt
ApaI
PspOMI
EcoRV
BsrGI
SpeI
10000
2000
SphI
p(CGCRSRRE)
11725 bps
CMV P
BsaAI
SnaBI
'InsIt
8000
Intron'
4000
MluI
NheI
SwaI
VSV-G
6000
'InsIt
DraIII
EcoNI
Poly A
RRE
CRS2
AgeI
BclI
BstBI
PpuMI
SanDI
BamHI
HpaI
PsiI
Eco52I
NotI

Fig 15E

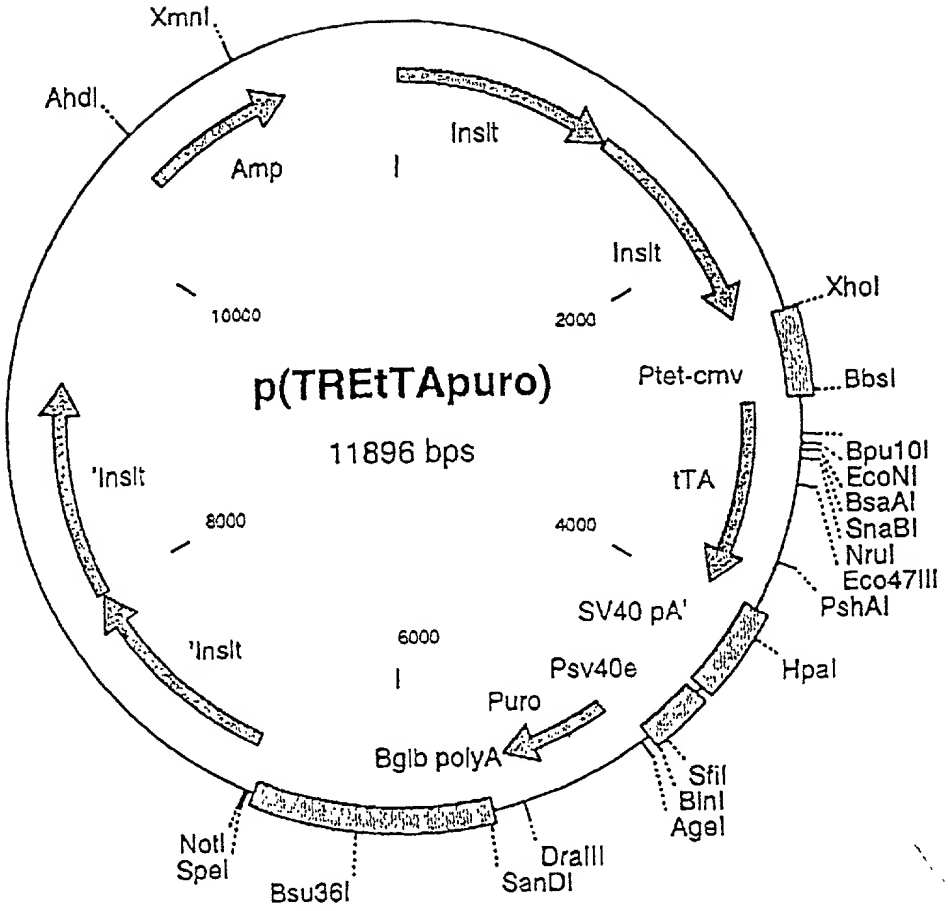

p(TREtTApuro)
11896 bps
Xmnl
Ahdl
Amp
InsIt
InsIt
Xhol
Bbsl
Ptet-cmv
tTA
Bpu10I
EcoNI
BsaAI
SnaBI
NruI
Eco47III
PshAI
Hpal
SV40 pA'
Psv40e
Puro
Bglb polyA
SfiI
BinI
AgeI
DraIII
SanDI
Bsu36I
NotI
SpeI
'InsIt
'InsIt
10000
2000
4000
6000
8000

Fig 15C

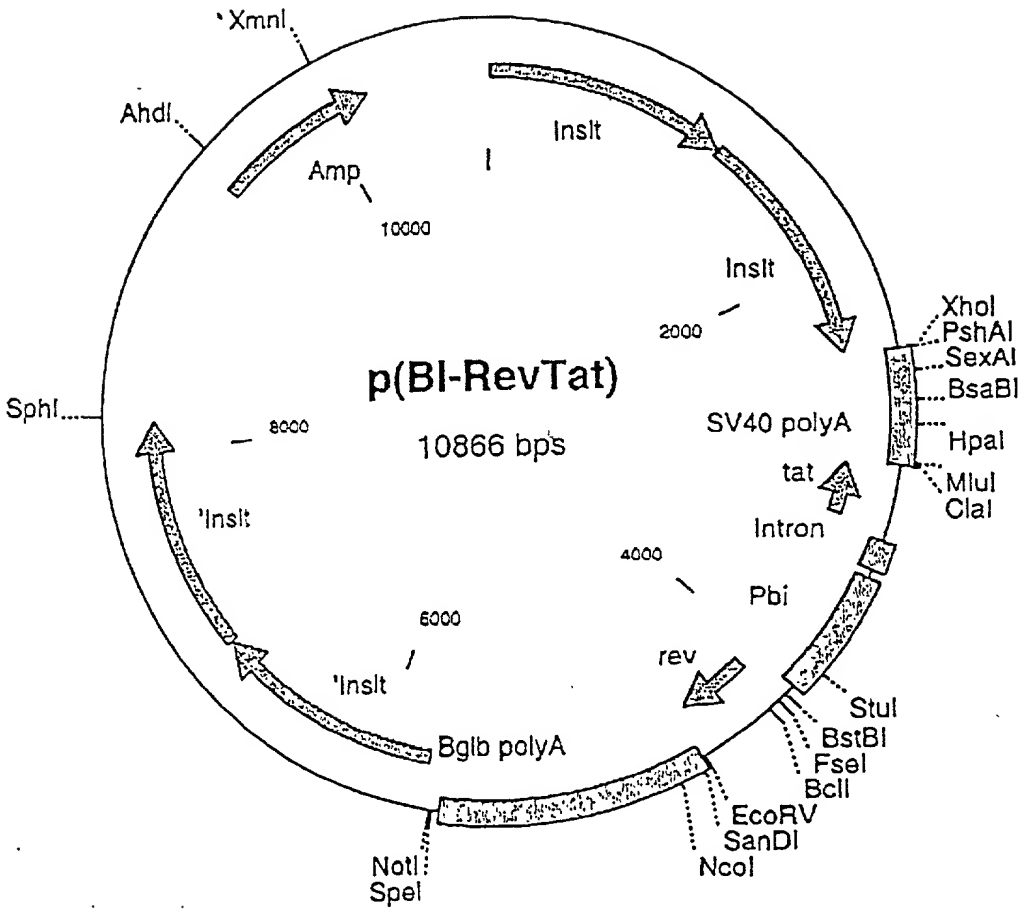

XmnI
AhdI
Amp
10000
InsIt
InsIt
2000
XhoI
PshAI
SexAI
BsaBI
HpaI
MluI
ClaI
p(Bl-RevTat)
10866 bp's
SV40 polyA
tat
Intron
4000
Pbi
rev
StuI
BstBI
FseI
BclI
EcoRV
SanDI
NcoI
SphI
8000
'InsIt
6000
'InsIt
BglB polyA
NotI
SpeI

Fig 15D

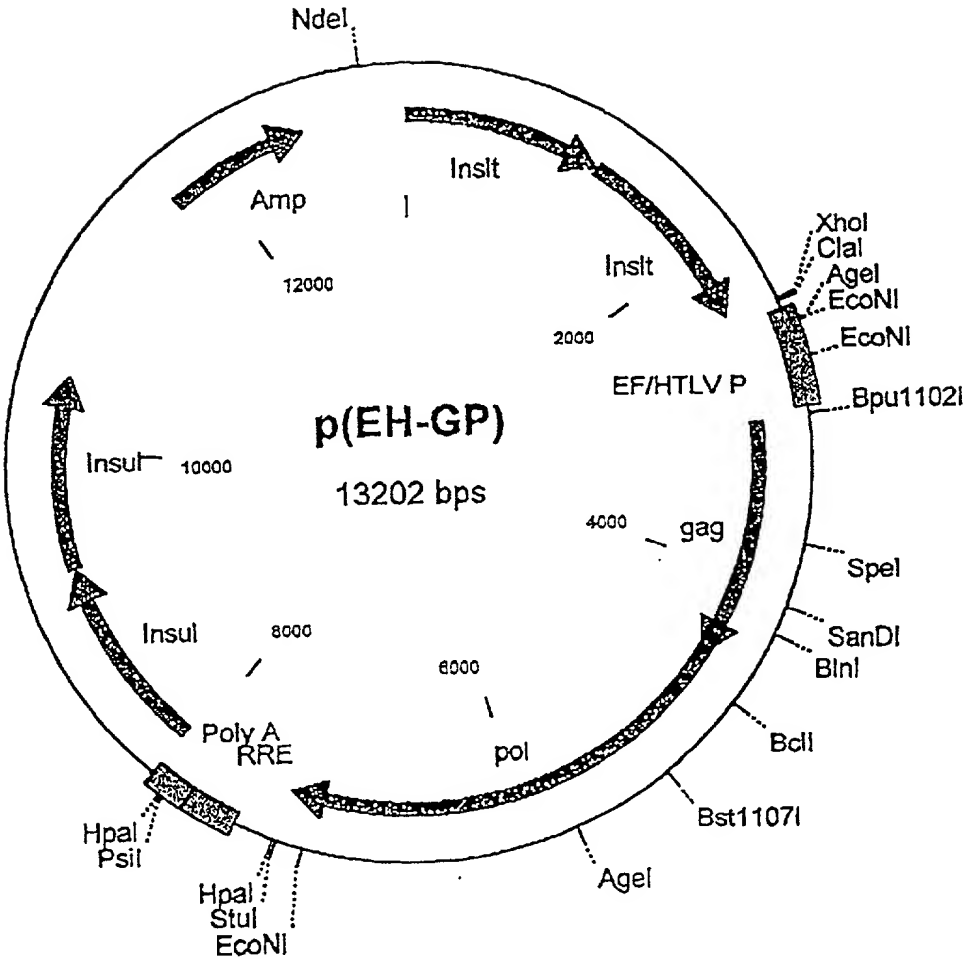

NdeI
InsIt
Amp
12000
InsIt
2000
XhoI
ClaI
AgeI
EcoNI
EcoNI
Bpu1102I
EF/HTLV P
p(EH-GP)
13202 bps
InsuI
10000
4000
gag
SpeI
SanDI
BlnI
InsuI
8000
6000
BclI
Poly A
RRE
pol
Bst1107I
HpaI
PsiI
AgeI
HpaI
StuI
EcoNI

Fig 15E

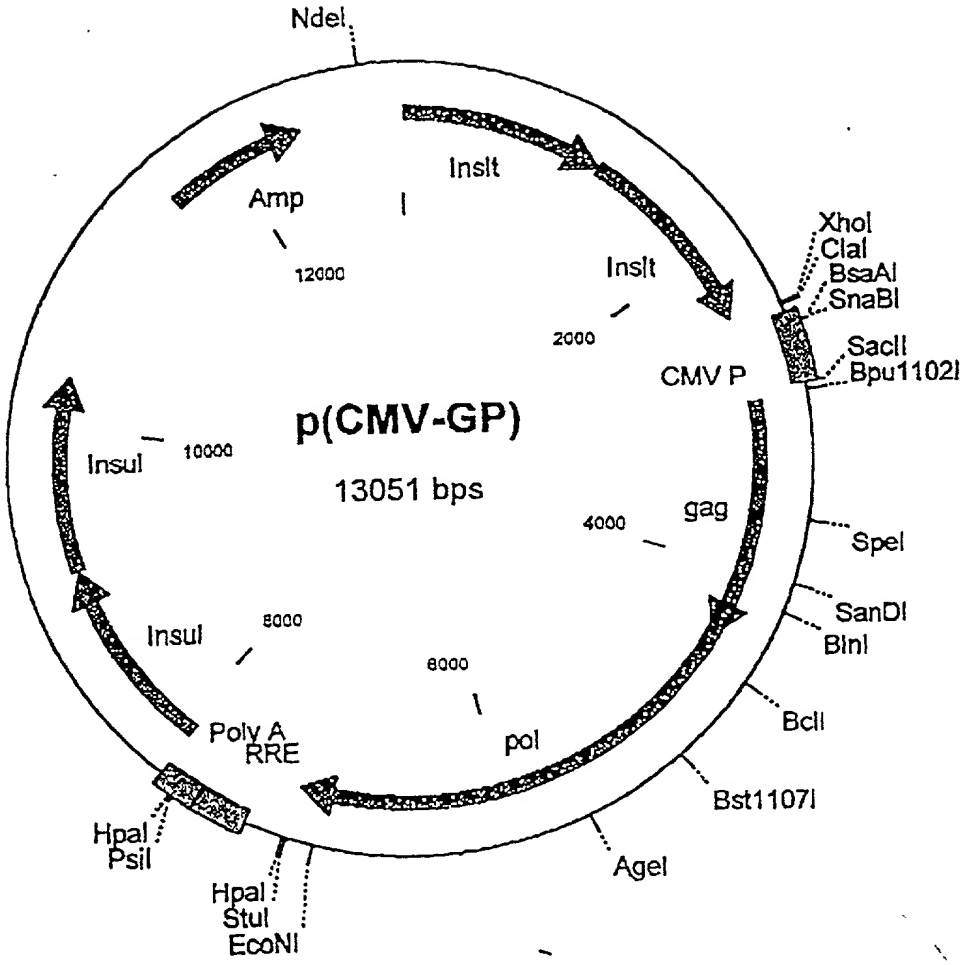

NdeI
InsIt
Amp
12000
InsIt
2000
XhoI
ClaI
BsaAI
SnaBI
SacII
Bpu1102I
CMV P
p(CMV-GP)
13051 bps
gag
4000
SpeI
SanDI
BlnI
BclI
Bst1107I
AgeI
pol
6000
Insul
8000
Insul
10000
Poly A
RRE
HpaI
PsiI
HpaI
StuI
EcoNI

Fig 15F

Rev dependent VSV-G constructs

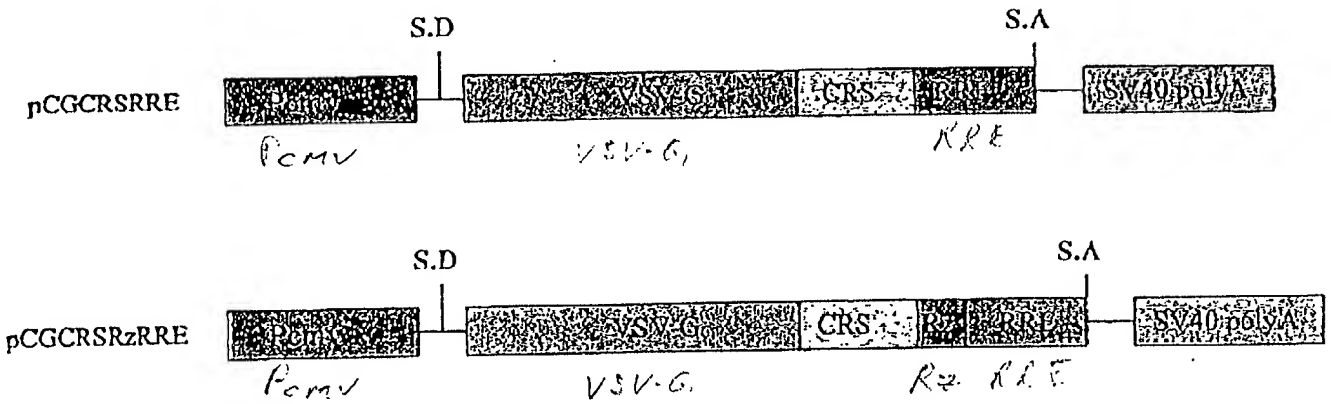
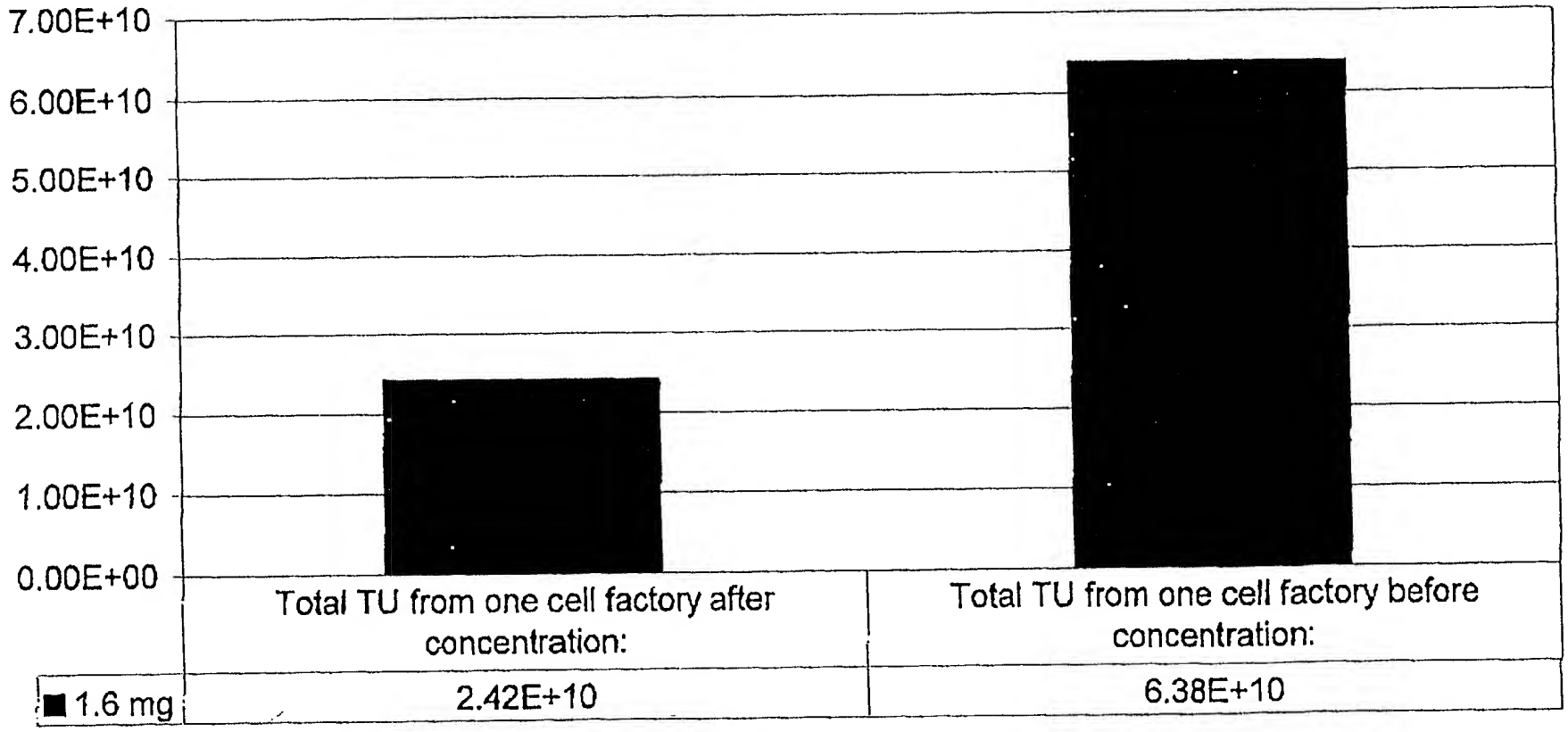


Figure 2

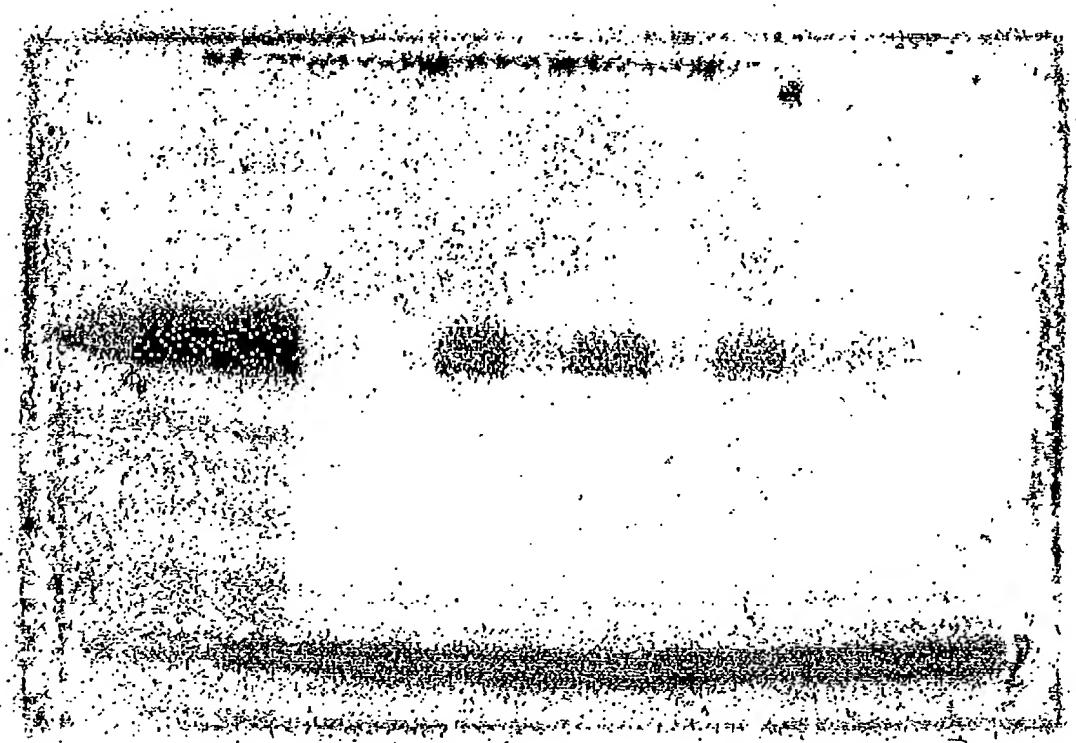
Yeild of pN1(cPT)GFP Vectors per Cell Factory before and after Concentration in HeLa-tat Cells.



AFTER CONCENTRATION

BEFORE CONCENTRATION.

Fig 17



+ = pCMV-Rev

− = pCI

G = β-globin SD

1M — HIV-1 major SD

H — Hammarskjold's SD Analog

1E — HIV-1 env SD

2E — HIV-2 env SD

REMOVE TETRACYCLINE TO INDUCE EXPRESSION OF VSV-G THAT IS REV DEPENDENT.

Fig 18

Influence of the Buffer on Vector Recovery after Storage for 3-5 Weeks at Different Temperatures

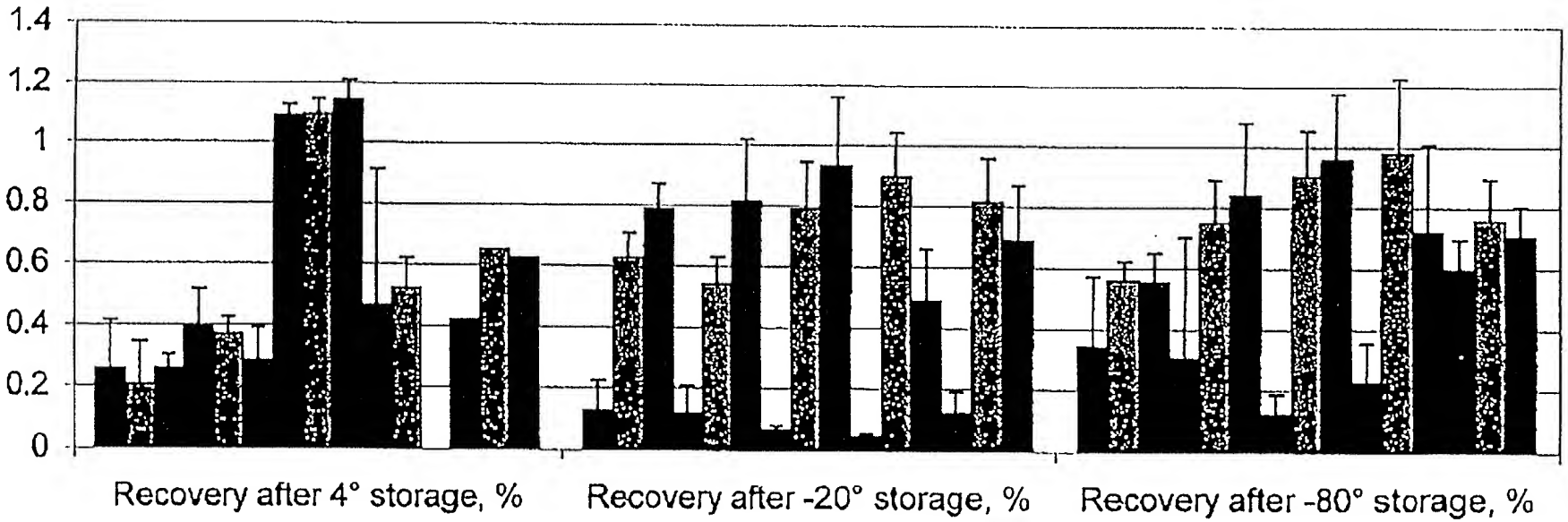
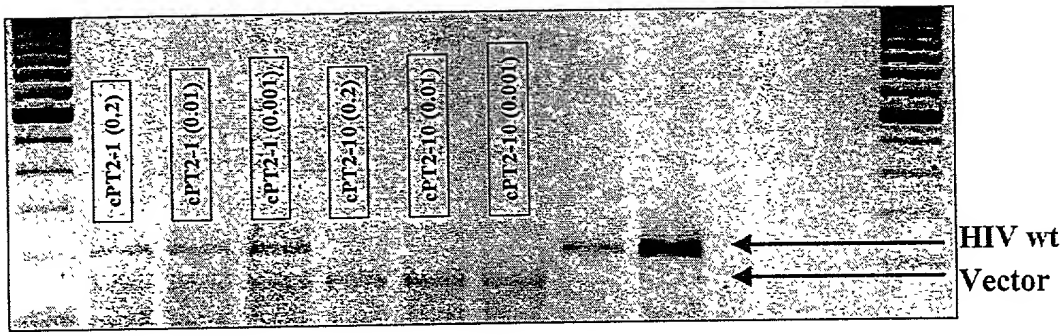


Figure 19


cPT2-1 (0.2)
cPT2-1 (0.01)
cPT2-1 (0.001)
cPT2-10 (0.2)
cPT2-10 (0.01)
cPT2-10 (0.001)
HIV wt
Vector